Effects of processing sorghum and millets on their phenolic phytochemicals and the implications of this to the health-enhancing properties of sorghum and millet food and beverage products

John R. N. Taylor and Kwaku G. Duodu

Correspondence to: Prof. J R N Taylor

Institute for Food, Nutrition and Well-being and Department of Food Science, University of Pretoria, Private Bag X20, Hatfield 0028, South Africa

Tel +27 12 4204296, Fax +27 12 4202839

e-mail: john.taylor@up.ac.za

Institute for Food, Nutrition and Well-being and Department of Food Science, University of Pretoria, South Africa

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Abstract

Sorghum and millet grains are generally rich in phytochemicals, particularly various types of phenolics. However, the types and amounts vary greatly between and within species. The food processing operations applied to these grains: dehulling and decortication, malting, fermentation and thermal processing dramatically affect the quantity of phenolics present, most generally reducing them. Thus, the levels of phytochemicals in sorghum and millet foods and beverages are usually considerably lower than in the grains. Notwithstanding this, there is considerable evidence that sorghum and millet foods and beverages have important functional and health-promoting effects, specifically antidiabetic, cardiovascular disease and cancer prevention due to the actions of these phytochemicals. Also their lactic acid bacteria fermented products may have probiotic effects related to their unique microflora. However, direct proof of these health-enhancing effects is lacking as most studies have been carried out on the grains or grain extracts and not the food and beverage products themselves, and also most research work has been *in vitro* or *ex vivo* and not *in vivo*. To provide the required evidence, better designed studies are needed. The sorghum and millet products should be fully characterised, especially their phytochemical composition. Most importantly, well-controlled human clinical studies and intervention trials are required.

INTRODUCTION

It is considered that sorghum and the millets are particularly nutritious cereal grains.[1] Further, sorghum and millet grains are also generally unusually rich in health-promoting phytochemicals, especially polyphenols,[2-5] and as a consequence that they are considered as being health-promoting foods".[6-8] However, as with most generalisations there are important qualifications. As summarised in Table 1, there are fundamental differences in the types and levels of phytochemicals between different species and between varieties of the same species of sorghum and millets. Phytochemical levels are also affected by cultivation environment.[2]

In relation to the concept that sorghum and millets are health-promoting foods, it is important to recognise that similar huge variations in phytochemical levels also occur in the more commonly cultivated cereals. Some varieties of wheat, maize and rice, notably those coloured black, blue, purple and red, contain high levels of levels of anthocyanin polyphenols[9] and total polyphenols.[10] and these can be in the same range or even higher than some sorghum and millet types.[10]

It is also important to take into consideration that some of the phytochemicals present in sorghum and millets are not necessarily health-promoting. Some varieties of sorghum and finger millet contain substantial levels of condensed tannins (proanthocyanidins and procyanidins) (Table 1). The condensed tannins in sorghum are generally considered as antinutrients, as they have been associated with adverse effects on dietary protein digestibility,[11,12] digestive enzyme activity[13] and mineral bioavailability.[13,14] Their inhibitory effects on starch digestion have, however, been suggested as being potentially

Table 1: Kernel structure, presence of particular phytochemicals and health related aspects of sorghum and the major millets

Name[a]	**Kernel structure**[127]	**Presence of tannins**[b3]	**Flavonoids**[b10]	**Phenolic acids**[b3]	**Significant phytochemicals**[b]	**Health related aspects**[c,d,e]
Sorghum *Sorghum bicolor* (L.) Moench	Caryopsis	Depends on sorghum type Type I (no pigmented testa) – none Type II (pigmented testa, pericarp generally chalky)– low levels Type III (pigmented testa , grain generally red/brown)– high levels	Levels depend on sorghum type High in black type, intermediate in red, low in white	Present all types at apparently relatively similar levels	3-deoxyanthocyanins[135] Policosanols[136]	Digestive amylase inhibition[b 15]. Evidence of slow starch digestion[c 25] Protein glycation inhibition[c 137]
Finger millet *Eleucine coracana* (L.) Gaertn.	Utricle	Depends on type[128] White and red (no pigmented testa) none Brown - yes - some varieties (with pigmented testa)	High levels[133]	Relatively low, intermediate levels[133]		Antihyperglycaemic effects[c,d 88,94] Anti-inflammatory[c 106]

Foxtail millet *Setaria italica* (L.) P. Beauv. subsp. *italica*	Utricle	Essentially none[129]	Intermediate levels[133]	High levels[133]		Antihyperglycaemic and hypolipidemic effects[c,d 89,97]
Pearl millet *Pennisetum glaucum* (L.) R. Br.	Caryopsis	Can contain low levels,[130] (more probably absent)[131]	Relatively low levels[133]	High levels[133]	*C*-glycosyl flavones (vitexin)[16]	Goitrogenic effects[d 19,20]
Proso millet *Panicum miliaceum* L. subsp. *miliaceum*	Utricle	Essentially none[129]	Relatively low levels[133]	High levels[133]		Serum cholesterol improvement[c 105]
Teff *Eragrostis tef* (Zuccagni) Trotter	Caryopsis	None[132]	Relatively low levels[134]	Relatively low levels		

[a]USDA Germplasm Resources Information Network http://www.ars-grin.gov/
[b]Actual levels of the various phytochemicals are given in the references
[c]*in vitro/ex vivo* study
[d]animal model study
[e]human study

beneficial in the prevention of type 2 diabetes and metabolic syndrome.[15] Also, pearl millet seems to uniquely contain flavonoid *C*-glycosyl flavones, especially vitexin,[16] which are goitrogens.[17,18] These glycosyl flavones have been implicated as a cause of the high incidence of goitre in certain communities in Sudan[19] and India[20] where pearl millet is a staple.

To date, most literature on the phytochemicals of sorghum and millets has been focussed on quantifying the levels and bioactive properties of those present in the grains,[4-6] probably with the assumption that that they will also be present and exhibit these health-enhancing activities in the sorghum and millet foods and beverages. However, for example, as shown in Table 1 the kernels of several of the major millet species are utricles (kernels with loosely attached sack-like seedcoats), which means that the “hull” must be removed when the grain is processed into foods. Further, even where the kernels are naked, as with sorghum, the grain is generally decorticated (debranned) to produce flour before further processing (generally hydrothermal processing) into food and beverage products.[21] Thus, an issue that has not been given adequate attention is whether foods and beverages produced from sorghum and the millets still contain substantial levels of phytochemicals.

Therefore this review examines the effects of food processing operations on the phenolic phytochemicals in sorghum and the major millets, and attempts to evaluate whether their food and beverage products are actually functional and have unique health-promoting properties due to the presence and action of these and other phytochemicals.

EFFECTS OF PROCESSING ON PHENOLIC PHYTOCHEMICALS IN SORGHUM AND MILLETS

The aim of processing as applied to grains is to transform the raw grains into finished products with good sensory and nutritional quality suitable for consumption. Processing affects the chemical constituents and physical properties of foods. As would be expected, processing has an influence on phytochemicals in sorghum and millets and this has a bearing on the potential health benefits that can be provided by the finished product. In this regard, the effect of various food processing methods on phytochemicals in sorghum and millets has become an important area of research. The effects of the four main processing methods applied to sorghum and millets to produce their food and beverage products: dehulling and decortication, malting, fermentation and thermal processing on their phenolic phytochemicals are discussed. It should be noted that these processing operations are generally performed in combination. For example, decortication and dehulling of the grain to produce a flour, which is subsequently wet cooked and then fermented into a sour porridge.

Dehulling and Decortication

These terms refer to the process whereby the outer layers of the grain, the hull where present (Table 1) and the pericarp are removed. This is done because they are coarse and generally unpalatable. Removal of the pericarp also removes antinutritional factors such as phytates and tannins and therefore can increase bioaccessibility of various nutrients. For instance, in the production of food products from tannin sorghum, decortication reduces astringency, improves digestibility and produces lighter coloured products,[21] which are generally preferred by consumers. Sorghum grains are normally decorticated by mechanically abrading off the outer layers of the kernel, which leaves clean endosperm that can then be milled into flour.[21] Millets are relatively more difficult to decorticate using abrasion primarily owing to their rather small size. In sorghum[22,23] and the millets,[24] flavonoid phenolic compounds other than tannins and waxes like the policosanols[136] are also concentrated in the outer layers of the grain. Therefore decortication, which removes these outer layers, also substantially reduces their content in the flours produced. These effects have been reported by various workers (Table 2).

Malting

Malting can be defined as the germination of grain in moist air under controlled conditions.[21] Sorghum is malted more extensively in comparison with the millets. Malted sorghum is used in the production of opaque beer in many countries in sub-Saharan Africa and increasingly in lager beer and malt beverages across the world.[25,26] Research into the effects of malting on phenolics in sorghum and millets has yielded mixed results. With sorghum[27-34] and millets[34-37] mostly decreases in phenolics have been reported (Table 2). However, there have also been reported increases with malting.[38-40] Various mechanisms have been proposed to account for these effects of malting on phenolic phytochemicals.

Leaching of phenolic compounds during steeping and germination has been proposed as one of the major modes by which phenolics are lost.[27,31-37] The aqueous environment that is present during steeping and germination facilitates solubilisation of phenolic compounds, which subsequently leach out into the steep liquor. For example, significant browning of supporting filter paper during germination of sorghum has been observed,[27] which was attributed to leaching of phenolic compounds from the seed coat of the grain.

Table 2: Summary of recent literature on effects of various processing methods on phenolic phytochemicals in sorghum and millets

Grain type	Processing method	Effect of processing and reference
	Dehulling and decortication	
Pigmented high tannin sorghums and white tan-plant sorghum	Decortication using a tangential abrasive dehulling device up to 6 min to produce different bran fractions	Earlier bran fractions had highest levels of total phenolics, tannins, 3-deoxyanthocyanins and antioxidant activity[23]
Tannin and non-tannin sorghums	Decortication for 6-8 min to 70-81% extraction rates using a Prairie Research Laboratory (PRL) dehuller	Decrease in total phenolics by 33% for non-tannin and by 77% for tannin sorghum and decrease in tannins by 79-92%. Decrease in ABTS radical scavenging capacity of tannin and non-tannin sorghums by 73-87%[56]
Tannin and non-tannin sorghums	Decortication to 70 and 90% extraction rates using PRL dehuller	Decortication to 70% extraction rate reduced total phenolic content of sorghum flours by 43-66% and ABTS radical scavenging by 25-89%. Decortication to 90% extraction rate reduced condensed tannin content of tannin sorghum by 12% and at 70% extraction rate, no tannin was detected[66]
Red and white non-tannin sorghums	Decortication by PRL dehuller for different times to obtain successive bran fractions	Decrease in phenolic content and DPPH scavenging activity with increasing time of decortications. Phenolic content of the endosperm was significantly lower than the rest of the bran fractions[138]
Pearl millet types	Mechanical dehuller	Reduction in total phenolic content by 49-51%[139]
Pearl millet	Decorticated using a hand grinder and air classification with a seed blower to separate fractions by size and density	Reduced total phenolic content, ORAC[a] value and hydroxyl radical scavenging capacity. Ferulic acid content reduced by 39% and *p*-coumaric acid by 52%[140]
Finger millet	Tempering with water for 10 min, milling and sieving to separate seed coat from refined flour	Refined flour had lower total phenolic content (0.8%) than wholemeal (2.3%). Seed coat fraction had highest total phenolic content (6.2%)[141]
Finger millet	Hydration and steaming followed by decortication with a horizontal carborundum disc mill	Decortication reduced polyphenol content by 75%[142]

Finger millet	Tempering with water and milling in a plate mill, sieving and separation of seed coat and flour fractions	Seed coat had highest total phenolic content of 13%. Refined flours had total phenolic contents of 3-4%[143]
Foxtail, proso, finger and pearl millets	Dehulling and decorticated using a hand grinder and air classification with a seed blower to separate fractions by size and density	Losses of total phenolics by 21%, 12-65%, 72%, and 2% for finger, foxtail, proso and pearl millets, respectively[62]
	Malting	
Red sorghums	Steeping and germination at 25°C	53% retention of total phenolics. Catechols and resorcinols were reduced to 54% and 44%, respectively[34]
Tannin sorghum	Steeping for 48 h and germination for 36 h	Increased levels of various phenolic compounds such as *p*-hydroxybenzaldehyde, *p*-hydroxybenzoyl alcohol, *p*-hydroxybenzoic acid and protocatechuic acid[144]
Red non-tannin sorghums	Steeping overnight and germination for 85 h	87% increase in total phenolics[40]
Pearl millet types	Steeping overnight and germination for 48 h	Soaking reduced total phenolics by 15% and germination decreased total phenolics by a further 73%[36]
Finger millet	Steeping for 24 h and germination for 96 h	Decrease in the major bound phenolic acids, caffeic acid, coumaric acid and ferulic acid by 45%, 42% and 48%, respectively[42]
Finger millet	Steeping and germination at 25°C	Reduction in total phenolics, catechols and resorcinols with 79%, 54% and 68% retention, respectively[34]
Finger millet	Steeping for 24 h and germination for up to 120 h	Loss of polyphenols by 44% after the first 24 h of germination and by another 40% over the next 48 h[37]
	Fermentation	
Sorghums	Natural fermentation at 37°C for 24 h	Reduction in tannin content by 15-35%[145]
Tannin sorghum	Lactic acid fermentation at 30°C for 1 day and alcoholic fermentation for 2 days	Reduction in proanthocyanidins by 54% during lactic acid fermentation and by 34% during alcoholic fermentation. Reduction in *p*-hydroxybenzaldehyde by 98% during lactic acid fermentation and by 92% during alcoholic fermentation[144]
Red sorghum	Fermentation at 30°C for 48 h to <pH 4	Reduction of total phenolics by 57%, catechols by 59% galloyls by 70%

		and resorcinols by 73%[14]
Red sorghums	Spontaneous lactic acid fermentation for 14 h	Increase in total reactive phenolic hydroxyl groups by more than 100%[40]
Tannin and non-tannin sorghums	Lactic acid fermentation to pH 3.6	Decrease in total phenolics and antioxidant activity[56]
Pearl millet types	Natural fermentation at 30°C for 14 h	Reduction of total polyphenols by 60% and 31%[146]
Finger millet	Natural fermentation at 37°C for 48 h	Reduction of total phenolics by 26-29% and tannins by 44-52%[58]
	Thermal processing	
Red sorghum	Boiling of whole grain	Reduction in total phenolics, catechols and resorcinols with 21%, 20% and 12% retention, respectively[34]
Tannin sorghum	Boiling of flour	Decrease in proanthocyanidin levels by 54%[144]
Red sorghum	Steaming soaked grains at 200-220°C for 20 min	Reductions in free vanillic acid and free *p*-coumaric acid, but increases in free ferulic acid and bound *p*-coumaric acid. Reductions in total phenolics, total flavonoids and procyanidin levels[63]
Tannin and non-tannin sorghums	Twin-screw extrusion cooking with feed moisture of 18% at 150- 160°C with 30-90 s residence time	Reduction in measurable total phenolics and tannins. Reduction in ABTS radical scavenging capacity by 83-87%[56]
Tannin and non-tannin sorghums	Baking sorghum dough into cookies at 180°C for 10 min	Reduced assayable condensed tannins by 95 and 96% in cookies made from 100 and 90% extraction rate flour, respectively[66]
Red sorghum	Roasting steamed grains at 150°C	Increases in levels of all detected phenolic acids except free vanillic and bound *p*-coumaric acid). Increases in total phenolics, total flavonoids and procyanidin[63]
Sorghums	Soaking grains in hot water, conditioning, steaming, tempering, partial drying and then flaking in a roller flaker and blistering in a fluidized bed roaster	Flaking reduced soluble polyphenols by 83%, bound polyphenols by 63% and total polyphenols by 75%. Blistering flaked sorghum increased soluble polyphenols by 39% and decreased bound polyphenols by 42%[69]
Pearl millet types	Wet cooking of whole and decorticated flour	Decrease in total phenolic content by 8-13%[139]
Pearl millet types	Heating of whole grains in hot air at 110°C	Decreased in total phenolics by 24%[36]
Finger millet	Boiling whole grain	Reduction in total phenolics, catechols and resorcinols with 60, 60 and 47% retention, respectively[34]

Finger, foxtail, proso and pearl millets	Boiling dehulled/decorticated grains	Reduction in total phenolic content of finger, foxtail, proso and pearl millets by 11-36%, 3%, 4%, and 4% respectively[62]

[a]Oxygen radical absorbance capacity

Another proposed mechanism by which germination of sorghum and millets may decrease phenolic compounds is that they enter the endosperm together with imbibed water during steeping and germination.[27,32,34] Within the endosperm, the polyphenols may bind with proteins and other macromolecules and become less extractable.[28,29,33,34] In this regard, it is perhaps incorrect to refer to a "loss" or "decrease" of phenolics due to germination. This is because by this mechanism, there is no loss of phenolics out of the system of the sorghum or millet grain as is the case with leaching. The phenolics stay within the grain system but become less extractable due to formation of complexes with macromolecules. For instance, condensed tannins are known to form irreversible complexes with sorghum kafirin prolamin protein.[41]

The activity of various enzymes that are mobilised during germination of sorghum and millets, also has an influence on phenolic content. It is suggested that polyphenol oxidase (PPO) is one of such enzymes.[29,33-35] Phenolic compounds are a substrate for PPO. Thus, the action of the enzyme would remove the phenolic substrate. Hydrolytic enzymes also play a role in the loss of phenolics during germination. Hydrolysis of tannin-protein and tannin-enzyme complexes has been mentioned as being responsible for removal of polyphenols during germination of pearl millet.[35] Enzymatic hydrolysis of polyphenols may bring about reductions in total phenolics and tannins during sprouting of pearl millet[35] and finger millet.[35] Hydrolytic enzymes that have been specifically mentioned are the esterases. These esterases are induced during germination and they act on phenolic acid esters linked to arabinoxylans and other non-starch polysaccharides.[37,42]

With regard to increases in polyphenols during sorghum malting, a progressive increase in tannin content over a five day germination period was attributed to solubilisation of tannin during the steeping process and migration of tannins to the grain outer layers, which was inferred from the observed browning of the germinated seeds.[38] However, it is worth noting that the suggested migration of tannins to the outer layers of the grain was not confirmed in this work. Other work reported an increase in total phenols, tannins, total leucoanthocyanin and total anthocyanin in four sorghum cultivars germinated for up to 96 h.[39] Tannin contents increased by up to 11 fold, while total phenols increased by between 7 and 14 fold. There were parallel increases in leucoanthocyanins and anthocyanins. These increases were attributed to *de novo* synthesis and polymerisation during germination. Other research reported an 87% increase in total phenolics in sorghum after malting.[40] The increase was attributed to losses of dry matter during the malting process (which may concentrate the phenolics) and hydrolysis of condensed procyanidins.

Fermentation

Many traditional sorghum and millet foods and beverages, especially in Africa, are fermented, either by lactic acid bacteria (LAB) alone or by a combination of LAB and yeasts, i.e. sourdoughs and traditional beer fermentations. There are several reviews describing these fermented products, which comprise flatbreads, doughs and dumplings, porridges, gruels, non-alcoholic beverages, opaque and cloudy beers.[43, 45-50] The metabolic activity of these microorganisms during the fermentation process, which involves various enzyme activities, has an effect on the chemical constituents of the food. Therefore microbial activity during fermentation of sorghum and millets determines the fate of phenolic compounds and functional and health-properties of the grains.

It appears that unlike malting, fermentation clearly reduces the levels of phenolics in sorghum and millets. For example, spontaneous fermentation (most likely lactic fermentation) of sorghum for 36 h was found to decrease tannins by 27%.[30] Further, spontaneous fermentation of grains that had been sprouted for 96 h decreased tannins by a further 57%.[30] The decrease in tannins was attributed to synergistic effects of sprouting and fermentation. It was suggested that the two processes produce enzymes that break down complexes to release free tannins which then leach out. Lactic acid fermentation for up to 36 h to produce *mahewu*, a non-alcoholic soured sorghum beverage, was found to decrease water soluble proanthocyanidins in tannin sorghum cultivars by up to 63%.[33] Similarly, fermentation was found to reduce assayable phenolic compounds in sorghum gruels, especially in gruels with added Power Flour (malted non-tannin sorghum).[51] The combination of fermentation with this addition of enzymes reduced total phenolics by 57%, catechols by 59%, galloyls by 70% and resorcinols by 73%. The authors outlined a number of reasons for the observed decreases in phenolics due to fermentation. These were: losses in phenolics due to the activity of PPO either from the cereal or the microflora,[53] the acidic environment during fermentation causing abstraction of hydride ions and rearrangement of phenolic structures,[53,54] and the acidic environment resulting cleavage of proanthocyanidins into flavan-3-ols which are subsequently oxidised to quinones.[53,54] Also, the fermentation process may have reduced extractability of phenolic compounds due to self-polymerisation and/or interaction with macromolecules such as proteins.[55]

It has been found that fermented slurries of whole and decorticated tannin sorghums had lower total phenols (measured using the Folin-Ciocalteu assay) and antioxidant activity (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid [ABTS] and di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium [DPPH] radical scavenging) compared to unprocessed whole and decorticated grains.[56] This highlights the generally observed trend of a positive correlation between total phenolic content and antioxidant activity of grains. It was suggested that this difference could have been due to changes occurring during the fermentation process that affected extractability of phenolics. The authors proposed that the observed effect of fermentation could be due to binding of phenolics to proteins and other components in the aqueous fermentation environment, making them less extractable,[57] or degradation of phenolics by microbial enzymes, e.g. PPO and peroxidase.[51]

With regard to fermentation of millets, a decrease in total polyphenol content of *rabadi*, a fermented pearl millet food, was found with increasing fermentation time at various temperatures.[52] Loss in polyphenol content was highest after 9 h fermentation. The authors suggested that reduction in polyphenols may be due to activity of PPO in the pearl millet grain or the fermenting microflora. Similarly, fermentation of finger millet for 48 h was found to reduce total phenolics by 26-29% and tannins by 44-52%.[58] The reduction in total phenolics and tannins in the first 24 h of fermentation corresponded with an increase in microbial population, which reached a maximum of 10^{10} cfu g^{-1} between 18 and 24 h and remained stable thereafter. It was suggested that release of fibre-bound tannins and PPO activity by fermenting microbes could explain the reduction in phenols due to fermentation.

Recent research has provided evidence that because of the antimicrobial nature of the phenolics in sorghum and millets, the LAB in their fermented products have the unusual ability to detoxify the phenolics.[59] When a type III tannin sorghum was fermented using binary combinations of *Lactobacillus plantarum* and *L. casei* or *L. fermentum* and *L. re*uteri isolated from a traditional Botswana sorghum fermented gruel, *ting*, it was found that phenolic acids, phenolic acid esters and flavonoid glucosides were metabolised.[147] Further, it was demonstrated that single strain cultures of the lactobacilli showed glucosidase, phenolic acid reductase, and phenolic acid decarboxylase activites, which contributed to the polyphenol metabolism. In related work, it was shown that these LAB, were different from those in wheat sourdough and this appeared to be due their resistance to phenolics and the fact that the LAB used glucose and not maltose as major carbon source.[60] The utilisation of glucose as a carbon source seems to be related to the fact that raw sorghum and millet grains lack β-amylase, the maltose producing enzyme.[61]

Thermal processing

Thermal processing is the most common method of processing sorghum and millets and is often applied in combination with one of more of the other processes. It involves various operations such as wet cooking, steam cooking, extrusion cooking (all hydrothermal) and baking and roasting (dry thermal processing). Thermal processing of sorghum and millets may increase or decrease in phenolic contents and antioxidant activities. Mechanisms proposed to account for these different effects include; release of bound phenolics from the food matrix, polymerisation and oxidation of phenolics, thermal degradation, depolymerisation of high molecular weight phenolics such as condensed tannins and production of Maillard reaction products.

Concerning wet cooking alone, boiling whole grain sorghum and finger millet in water for 15 min reduced the total phenolic content of sorghum by 79% and finger millet by 40%, respectively.[34] Retention of catechols and resorcinols in sorghum after cooking was 20% and 12%, respectively. With finger millet, retention after cooking was 60% and 47% for catechols and resorcinols, respectively. These findings were attributed to thermal degradation of the phenolics, leaching of phenolics into the cooking media, and migration of phenolics into the endosperm with the imbibed water during cooking.[34] It was suggested that these phenolics then formed complexes with macromolecules such as proteins and their extractability was decreased. Similarly, boiling various dehulled finger millet grains in water for 30 min reduced their total phenolic content by 11-36%.[62] It was suggested that degradation of phenolics upon heat treatment and leaching of phenolics into the endosperm to form complexes with proteins and other macromolecules with reduced extractability could contribute to the observed decreases.

Steaming whole grain sorghum (previously soaked in water for 1 h and dried to constant weight at 50^{o}C) caused losses of free vanillic acid and free *p*-coumaric acid, but increased free ferulic acid and bound *p*-coumaric acid.[63] Soaking and steaming also reduced total phenolics, total flavonoids and procyanidin levels. These losses were attributed to leaching of some soluble conjugated phenolic acids and other water-soluble phenolic compounds into the soaking water and oxidative degradation of phenolics by steaming.

Extrusion cooking has been reported to reduce measurable total phenolics and tannins and ABTS radical scavenging activity in both milled whole grain and decorticated tannin sorghums.[56] The authors suggested that the specific effect of extrusion may be dependent on feed moisture during extrusion process. High feed moisture (≥ 18%) could promote phenolic and tannin polymerisation, reduce their extractability and reduce antioxidant activity.[64] This may potentially be an indication of the polymerisation of phenolics through a mechanism of oxidative coupling under the given extrusion conditions. With lower feed moisture (< 15%), high shear and high temperature conditions of the extrusion process may depolymerise condensed tannins and convert them into lower molecular weight oligomers that are more extractable.[65] The depolymerisation of higher oligomeric polyphenols into lower molecular weight variants may not necessarily always lead to greater extractability. Similarly, protein sequestration could occur during extrusion cooking and this could promote formation of insoluble protein-polyphenol complexes which is a well-known phenomenon.[41]

Concerning dry heat treatments, baking condensed tannin sorghum flours into cookies was found to reduce assayable condensed tannins by 95 and 96% in cookies made from 100 and 90% extraction rate flours, respectively.[66] It was suggested that sorghum proteins may have formed insoluble complexes with the condensed tannins[41] during dough preparation and baking, thus reducing their extractability. When calculated on the basis of the proportion of flour in the cookies, total phenolic content and antioxidant activity of the cookies were actually higher than in the in flours. It was suggested that the formation of Maillard reaction products, which possess reducing properties,[67] and release of bound phenolic acids from cell walls during baking[68] may have been responsible for the apparent increase in total phenolics and antioxidant activity on baking. On the other hand, the more severe dry heat treatment of roasting whole grain sorghum (previously steamed and then dried to constant weight at 50^{o}C) was found to increase phenolics in sorghum.[63] Roasting caused increases in levels of all the detected phenolic acids (except free vanillic and bound *p*-coumaric acid). It also increased total phenolics, total flavonoids and procyanidin levels. The increases were attributed to release of bound phenolics from cell walls, degradation of conjugated polyphenolics, e.g. tannins, at high temperature to simple phenolics and overall increased in extractability of phenolic components after roasting.

With regard to thermal treatment in combination with other processing operations, when sorghum grain were taken through a flaking and blistering process, which involved soaking the grains in hot water, conditioning, steaming, tempering, partial drying, flaking in a roller flaker and blistered in a fluidized bed roaster, flaking reduced soluble polyphenols by 83%, bound polyphenols by 63% and total polyphenols by 75%.[69] The decrease in polyphenols was attributed to loss of bran layers from the whole grain during flaking. Blistering the flaked sorghum increased soluble polyphenols by 39% and decreased bound polyphenols by 42%, while there was no change in total polyphenols. The increase in soluble polyphenols during blistering was attributed to liberation of bound polyphenols and higher extractability due to changes in starch structure on dry heat treatment during the blistering process. Similar results regarding soluble polyphenols were observed with pearl millet.[69] Similarly, it has been reported that wet cooking flour prepared from sprouted sorghum grains, followed by fermentation for 36 h synergistically reduced tannins in sorghum.[30] The flour from grains sprouted (malted) for 72 h and 96 h both had a tannin reduction of 49% after cooking and fermentation. It was suggested that the reduction in tannins was due to the activity of fermentation organisms and protein denaturation. The authors hypothesized that cooking and fermentation broke down tannin-enzyme and protein-tannin complexes and released free tannins which were subsequently leached out.

Potential functional and health-promoting properties associated with the phenolic and other phytochemicals in sorghum and millet include anti-metabolic syndrome effects, specifically anti-diabetic, anti-inflammatory, anti-hypertensive and cardiovascular disease (CVD) prevention, and also anti-cancer[5,6] and microbial disease prevention (prebiotic and probiotic effects).[47,50] Next, the evidence for these properties in sorghum and millet food and beverage products will be examined.

FUNCTIONAL AND HEALTH-PROMOTING PROPERTIES OF SORGHUM AND MILLET FOODS AND BEVERAGES IN RELATION TO THEIR PHYTOCHEMICALS

Traditional sorghum and millet foods and beverages include whole grain snacks, "rice", semolina, couscous, doughs and dumplings, flatbreads, porridges and gruels, non-alcoholic beverages and opaque and cloudy beers. Non-traditional products include ready-to-eat snack foods and gluten-free pasta, noodles, baked products, malted beverages, lager and stout beers. The methods of preparation of these products, which invariably include combinations of the four operations discussed, have been extensively reviewed.[8,24,25,43,70-76] Here, *in vivo* research work which concerns the health-promoting properties of sorghum and millet food and beverage products in relation to the phytochemicals present, is reviewed. Where such work does not exist, relevant *in vivo* work on grains and extracts and *in vitro* work on food products are reviewed.

Anti-diabetic effects

There is good evidence that wet cooked sorghum foods such as porridges exhibit slower starch digestion than similar products than other cereals.[77] This is primarily due to the endosperm protein matrix, cell wall material and condensed tannins (if present in the particular variety and if not removed by decortication) inhibiting enzymatic hydrolysis of the starch. Polypeptide disulphide bond cross-linking involving the kafirin prolamin proteins in the protein matrix that envelops the starch granules in the corneous endosperm is probably the major factor that limits starch digestion.[78-80]

The evidence of specific inhibition by sorghum tannins on starch digestibility seems to be contradictory. *In vitro* work showed that bran [81] and bran extracts[15] from tannin sorghum decreased the starch digestibility, estimated glycaemic index and increased resistant starch (RS) of sorghum endosperm porridges. In apparent contrast, it has been found that with cooked whole grain sorghum flours (whether freshly cooked or stored), there was no correlation between tannin content, nor tannin molecular weight and rapidly digestible, slowly digestible, or RS.[82] However, other work shows that sorghum tannins do actually interact strongly with starch and decrease starch digestibility.[83]

There is also evidence from animal model studies that sorghum phenolics exert more subtle antidiabetic metabolic effects than simply inhibiting starch digestion. Solvent extracts (the common procedure used for selectively extracting phenolics) from sorghum have been shown to inhibit hepatic gluconeogenesis (glycogen synthesis) in streptozotocin-induced diabetic rats.[84] Sorghum extracts were also found to increase insulin sensitivity in mice fed high fat diets.[85] The research indicated that this was as result of increased adiponectin (a hormone regulating glucose levels and fatty acid breakdown) and decreased tumour necrosis factor-α, via overexpression of peroxisome proliferator-activated receptor gamma (a regulator of fatty acid storage and glucose metabolism) from apidose tissue. A criticism of these works is that the phenolics in these extracts were not measured so the levels may not have been similar to those in food products.

With regard to the millets, there is also good evidence that finger millet condensed tannins are powerful inhibitors of the key digestive amylase enzymes, pancreatic α-amylase and α-glucosidase.[86] Similar to the evidence with sorghum, finger millet phenolic-rich seed coat, which is invariably removed during processing, was found to exhibit very positive, more subtle, antidiabetic effects when fed to streptozotocin-induced diabetic rats.[87] In addition to reduced fasting hyperglycaemia, there was lower lens aldose reductase activity, lower serum advanced glycation end products, lower glycosylated haemoglobin levels and reduced cataracts (all indicators of reduced glucose-protein reactions). Further, there was partial reversal of kidney damage and improved body weights compared to the control group. Whole grain finger millet flour has also been found to be antihyperglycaemic and reduce collagen glycation in rats with diabetes induced by alloxan.[88] Regarding other millets, an aqueous extract of foxtail millet was shown to exert strong antihyperglycaemic effects in streptozotocin-induced diabetic rats.[89] It should be noted that such aqueous extracts will contain many other substances in addition to phenolics.

A key issue is do the various effects translate into to a lower glycaemic response in human subjects consuming sorghum and millet foods? An early and comprehensive study involved feeding three whole grain sorghum and equivalent “dehulled” (debranned) sorghum products and equivalent wheat and rice products consumed in India *missi roti* (fried flatbread), *upma* (boiled semolina) and *dholka* (fermented steamed product) to six non-insulin dependent patients.[90] All the whole grain sorghum products gave a much lower glycaemic response than the debranned sorghum products. Notably, however, there were no differences in glycaemic response between the debranned sorghum products and their wheat or rice equivalent. A weakness in the study was the sorghum used was not characterised in terms of variety or phenolic content.

There have been several investigations involving human subjects into whether finger millet has hypoglycaemic effects, going back to 1957[24,91]. The results from some of the more reliable studies with respect to grain and food product characterisation and comparisons are summarised. The effect of *roti* (flatbread) and dumplings made from finger millet, rice and sorghum on the glycaemic response in non-insulin dependent diabetic (NIDD) and normal subjects was measured.[92] In NIDD subjects, the finger millet dumpling elicited a higher glycaemic response than the sorghum and rice dumplings and all the *rotis* made from the different cereals. In other work, porridges prepared from wheat, decorticated (debranned) finger millet, popped and expanded rice were given to healthy subjects and their glycaemic indices (GIs) measured. [93] Interestingly, the wheat porridge elicited by far the lowest GI (55±9) and that of porridge from decorticated finger millet (93±7) was not much less than that of the rice products (105±6 and ±109±8). However, it has been found that noodles made from a composite flour of finger millet and refined wheat elicited a lower GI (45) than refined wheat noodles (63).[94] Similarly, the GIs of refined wheat, wheat-finger millet and wheat-foxtail millet composite breads[95] and composite wheat-foxtail millet biscuits [96] were measured when given to healthy subjects. Both types of millet-wheat composite breads had lower GIs than wheat bread, 41-43, 50 and 68, respectively[95]and the finger millet-wheat composite biscuits had somewhat lower GI than the wheat biscuits, 51 versus 68.[96] However, it is not evident from these latter three studies whether the millets were decorticated or not. Probably more significantly with respect to determining whether foxtail millet has any hypoglycaemic effect, a cross-over randomised clinical trial was undertaken where foxtail millet-wheat composite biscuits and foxtail millet-chickpea composite *burfi* (a sweet) were fed to type 2 diabetics.[97] It was found that both products resulted in moderate long-term improvement in glycaemic response but did not affect glycosylated haemoglobin levels.

Despite all this research, a recent review concluded that many of the GI studies on antidiabetic effects of finger millet and other millets have been flawed due to out-dated methodology and it is difficult to draw meaningful interpretations.[24]

Anti-inflammatory effects and CVD prevention

Meta-analysis of cohort studies (analysis of several prospective studies of the association of risk factors with disease outcomes) has shown that there is a consistent inverse association between dietary intake of whole grains, including sorghum and millets in incident CVD.[98] However, data directly on the impact of consumption of sorghum and millet products on CVD seem to be non-existent, but there is some evidence that they affect the conditions that cause CVD or that are associated with CVD. With regard to whole grain consumption effects, organic solvent extracts of brans from black polyphenol-rich and tannin sorghum were found to reduce the oedema (swelling) in chemically induced inflamed ears of mice.[99] The authors attributed this to inhibition of the pro-inflammatory cytokines interleukin-1β and tumour necrosis factor-α, which regulate the inflammatory response and immune cells. In support of the hypothesis that the effects were due to polyphenols and tannins, bran extracts from white or red and bronze pericarp sorghums, or oat, rice and wheat did not elicit anti-inflammatory effects.[99] Similar anti-inflammatory effects were found with bran extracts from golden gelatinous sorghum.[100] Importantly, the extracts were found to be nontoxic by a rat animal model acute toxicity test. An aqueous extract of proso millet (the composition of which was not described but likely rich in phenolics) was found to reduce blood triglyceride levels, liver lipid accumulation and total cholesterol levels in genetically obese mice.[101] The authors presented evidence that these effects were related to regulation of hepatic lipogenesis and lipolytic gene expression and also inhibition of release of cytokines and chemokines.

Potential effects of sorghum and millets on conditions causing or associated with CVD do not seem to be solely due to phenolics. Lipid extracts from red non-tannin sorghum were found to reduce cholesterol absorption and non-high density lipoprotein (HDL) cholesterol concentrations when fed to hamsters.[102] The authors suggested that plant sterols were responsible for reducing cholesterol absorption and that policosanols, which are present in sorghum,[136] may have inhibited cholesterol synthesis. Later work by the group indicated that the sorghum lipids strongly affected the type of gut microflora, which in turn may have improved cholesterol homeostasis,[103] presumably through short chain fatty acid metabolism by the microflora. Further, a protein concentrate from foxtail millet was found to increase plasma HDL cholesterol and adiponectin levels when fed to genetically type 2 diabetic mice.[104] Similar results were obtained when proso millet protein concentrate was fed to rats.[105]

With regard to research on effects of consumption of actual grains, as opposed to extracts and grain components, dietary induced hyperlipidemic rats were variously fed diets containing raw whole finger millet, proso millet and sorghum and white rice (details of grains not given) with interesting results.[106.] Serum triglyceride levels were substantially lower in the two millet groups than the sorghum and white rice groups. Levels of C-reactive protein (an indicator of inflammation) were lower in the finger millet group than the other groups. The group fed sorghum had significantly higher serum-, total-, HDL- and low-density lipoprotein-cholesterol levels than the other groups. Oxidative status was not affected in any of the groups.

The role of oxidative stress in the development of disease is important in this regard. Oxidative stress refers to the condition where there is an imbalance between the generation of reactive oxygen or nitrogen species (products of cellular redox processes within the body) and the activity of the natural antioxidant defence systems of the body.[148] Severe oxidative stress is implicated in various diseases, including cardiovascular disease, cancer and type 2 diabetes. Phenolics in general and in grain foods are believed to reduce oxidative stress through various mechanisms, including: suppression of the formation of reactive oxygen species either by inhibition of enzymes or chelating trace elements involved in free radical production; scavenging reactive oxygen species; upregulating or protecting antioxidant defence systems (such as the glutathione radical scavenging system) and by increasing plasma uric acid levels, which has reducing and free radical scavenging activities.[149,150]

Anti-cancer

Two frequently quoted studies, one across many countries including several parts of Africa[107] and one in China[108] indicated that consumption of sorghum and millets was associated with a lower incidence of oesophageal cancer. However, it should to be pointed out that the former suggested that high rates of oesophageal cancer were associated with micronutrient deficiency. As reviewed,[5,6] there are a number of *ex vivo* (cancer cell line) studies and Phase II enzyme (endogenous enzymes that inactivate potential carcinogenic metabolites through conjugation) induction studies showing that phenolic extracts from sorghum and millets exhibit potential anti-cancer properties.

However, as is the case with CVD research, *in vivo* data are much more limited and there are essentially no food product data. When rats were fed red sorghum (presumably non-tannin), millet (species not given), brown (whole grain) rice or potatoes was found to result in much lower levels of chemical induced oesophageal cancer than maize, wheat, polished (debranned) rice, bananas and interestingly bird-resistant (tannin) sorghum.[109] In support of the findings from the same authors' epidemiological study[107], supplementation of the maize and wheat diets with vitamins and minerals significantly reduced the numbers of oesophageal tumours.

Much more recently, in apparent contrast, it has been shown that mice injected with procyanidin-rich (tannin) extracts from sorghum bran showed inhibited lung cancer tumour growth and metastasis formation when lung cancer cells were subcutaneously injected.[110] This appeared to be as a result of suppression of production of the Vascular Endothelial Growth Factor, a signal protein that stimulates vasculogenesis and angiogenesis. Similarly, injected sorghum phenolic extracts were found supress tumour growth and inhibit metastasis to the lung in mice given breast cancer xenografts.[111] The data indicated that the phenolic extracts modulated the Janus kinase/Signal Transducer and Activator Transcription (Jak/STAT) signalling pathway, which is involved in gene promotion causing DNA transcription.

Prebiotic, probiotic and antimicrobial effects

An important property of fermented sorghum and millet products is that because of their low pH, typically below pH 4.0, they are generally microbiologically safe.[112] Early research revealed that the growth of Gram negative pathogens (*Escherichia coli*, *Campylobacter jeuni*, *Shigella flexneri* and *Salmonella typhimurium*) was strongly inhibited by the low pH, < pH 4.0, of naturally fermented sorghum.[113,114] Related work also suggested that the Gram positive *Staphylococcus aureus* was additionally inhibited due to bacteriocin action.[113] More recent research has provided evidence that fermented sorghum and millet products can have both prebiotic and probiotic activities and that the microorganisms involved can produce bacteriocins.

Prebiotics are indigestible, but fermentable carbohydrates that selectively stimulate bacterial groups in the colon that are beneficial to the human host.[115] It has been found that sorghum sourdoughs fermented with a probiotic stain of *Weisella cibaria* produced substantial levels of prebiobic isomaltooligosaccharides (59 g kg^{-1} dm).[116] Dietary fibre fractions from both pearl millet and foxtail millet have also been shown to act as excellent substrates for the probiotic bacteria *Lactobacillus acidophilus, L. rhamnosis*, *Bifidobacterium bifidum* and *B. longum.*[117] Acetate, propionate and butyrate in descending order were the major short chain fatty acids produced by both the lactobacilli and the bifidobacteria.

Probiobics are viable microorganisms that promote or support a beneficial balance of the autochthonous (indigenous) microbial population in the gastrointestinal tract.[115] The probiotic potential of 152 LAB isolated from 12 different samples of traditional fermented pearl millet gruel, *ben-saalga* (prepared from milled whole grain flour), produced in Burkina Faso was analysed by genetic screening using 16S rRNA gene sequencing.[118] Genes associated with bile salt tolerance (indicative of potential to survive in the human intestine) were widely distributed. However, only a limited set of isolates, mainly those belonging to *L. fermentum* could tolerate the low pH, pH 2, indicative of gastric survival. Yeasts isolated from traditional fermented sorghum and millet products have also been found to have probiotic potential. Strains of *Candida krusei*, *Kluyveromyces marxianus*, *Candida rugosa* and *Trichosporon asahii* isolated from *fura*, a Ghanaian fermented pearl millet dough, were found to increase the transepithelial electrical resistance (TEER), an indication of improved gastrointestinal tract barrier properties, in an intestinal cell model system, polarized human intestinal CaCo-2 cells.[119] However, surprisingly, in general they decreased the TEER in another mammalian cell model system, porcine intestinal IPEC-J2 cells.

Bacteriocins are generally considered as antibacterial proteins that are produced by LAB.[120] A traditional Indian fermented finger millet food, *koozh* has been found to contain a bacteriocin producing strain of *Enterococcus faecium.*[121] The bacteriocin, which was highly stable against proteases, inhibited the growth the food-borne pathogens including *Leuconostoc mesenteroides* and *Listeria monocytogenes* and also had substantial bactericidal activity. Similarly, strains of *L. reuteri* and *Pediococcus acidilacti* isolated from *fura*, the fermented pearl millet dough, have been shown to produce bacteriocins with inhibitory activity against a wide range of pathogens.[122]

Research also indicates that LAB isolated from *ogi*, a fermented sorghum gruel from Nigeria, can produce bacteriocins with high fungicidal activity.[123]

With regard to specific effects of sorghum and millet phenolics, it has been found that a methanolic extract of the "husk" of brown finger millet (presumably a tannin-rich extract) exhibited higher antimicrobial activity against *Bacillus cereus* and *Aspergillus flavus* than an extract from whole grain flour.[143] Similarly, a methanolic extract from a type III tannin sorghum was found to exhibit higher antimicrobial activity against *B. subtilis* and *Listeria monocytogenes* than extracts from white non-tannin sorghum.[147] Importantly, it was found that LAB fermentation of the sorghum by lactobacilli isolated from traditional fermented sorghum porridge did not reduce the levels antimicrobial activity. As explained, it appears that such lactobacilli can detoxify the phenolics, which enables them to grow,[59,147] whereas the growth of other microorganisms is suppressed.

An important question, however, is whether the consumption of naturally fermented sorghum and millet products is actually beneficial to human health. Research has been conducted into the use of traditional sorghum and millet foods for children suffering from diarrhoea. Children in Tanzania, after rehydration treatment, were fed different types of cereal porridges: conventional, high energy (amylase treated) or *togwa*, a fermented sorghum gruel.[124] Those receiving *togwa* showed better improvement in intestinal barrier function. More recently, another study involved feeding *koko sour water*, a live fermented pearl millet gruel, to infants suffering from diarrhoea who had been brought to health clinics in Ghana.[125] The microflora in *koko sour water* had previously been shown to have some probiotic potential with the dominant LAB being *Weisella confusa* and *L. fermentum*.[126] However, no effect on the infants' stool frequency or consistency, or duration of diarrhoea was found.[125] It should be noted, however, that most of the infants also received antibiotic treatment. Notwithstanding this, according to the authors, any effect in reducing diarrhoea by using spontaneously fermented cereal foods is yet to proven.[125] A review of African fermented foods reached a similar but more general conclusion that there was an absence of firm evidence about their probiotic induced health benefits due in large part to a paucity of properly designed research studies.[47]

CONCLUSIONS

Sorghum and millet grains are generally rich in phytochemicals, particularly various types of phenolics. However, the types and amounts vary greatly between and within species, and also with cultivation environment. Importantly, food processing dramatically affects the levels of these phenolic phytochemicals in sorghum and millets. Dehulling and decortication, and fermentation reduce the levels. Generally, malting also reduces the levels. The effects of thermal processing are complex, as release of bound phenolics and depolymerisation of polyphenols can take place. Thus, the levels of phytochemicals in sorghum and millet foods and beverages are generally considerably lower than in the grains. With regard to whether the hypothesised functional and health-promoting effects of sorghum and millet food and beverages in relation to their phenolics and other phytochemicals are proven facts, the above-mentioned cautions about the proposed antidiabetic effects of millets[24] seem to also apply to research into the potential effects of CVD and cancer prevention. Concerning LAB fermented sorghum and millets products, they may have probiotic effects related to the unique properties of their microflora. Thus, while collectively the research evidence that the phenolics and other phytochemicals in sorghum and millet products do exhibit health-enhancing effects is strong, direct proof is lacking.

As indicated, there is a general need for better designed studies. When the health-enhancing properties of sorghum and millets are investigated the grains and the food and beverage products being studied need to be fully characterised, especially with regard to levels and types of phytochemicals. In animal model studies, food and beverage products as consumed should be used and not just raw flours or extracts as at present. Most importantly, well-controlled human clinical studies and intervention trials are required.

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