Increasing the utilisation of sorghum, millets and pseudocereals: Developments in the science of their phenolic phytochemicals, biofortification and protein functionality

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Abbreviations: ABC, ATP-Binding Cassette; ABS, Africa Biofortified Sorghum; ACE, Angiotensin-1 Converting Enzyme; DDGS, distillers dried grain and solubles; FAN, free amino nitrogen; GM, Genetically Modified; GBSS, Granule-bound starch synthase; HDL, high density lipoprotein; ICRISAT, International Crops Research Institute for the Semi-Arid Tropics; LDL, low density lipoprotein; LKR, lysine ketoreductase; MIK, myo-inositol kinase; MRP, Multidrug resistance-associated protein; QTL, Quantitative Trait Locus; RNAi,

RNA Interference technology; TBARS, thiobarbituric acid reactive substances; TNF, Tumour Necrosis Factor.

Abstract

There is considerable interest in sorghum, millets and pseudocereals for their phytochemical content, their nutritional potential and their use in gluten-free products. They are generally rich in a several phenolic phytochemicals. Research has indicated that the phenolics in these grains may have several important health-promoting properties: prevention and reduction of oxidative stress, anti-cancer, anti-diabetic, anti-inflammatory, anti-hypertensive and cardiovascular disease prevention. However, increased research on the actual health-promoting properties of foods made from these grains is required. Biofortified (macro- and micronutrient enhanced) sorghum and millets are being developed through conventional breeding and recombinant DNA technology to combat malnutrition in developing countries. Enhanced nutritional traits include: high amylopectin, high lysine, improved protein digestibility, provitamin A rich, high iron and zinc, and improved mineral bioavailability through phytate reduction. Some of these biofortified cereals also have good agronomic characteristics and useful commercial end-use attributes, which will be important to their adoption by farmers. Knowledge of the structure of their storage proteins is increasing. Drawing on research concerning maize zein, which shows that it can produce a visco-elastic wheat-like dough, it appears that the storage proteins of these minor grains also have this potential. Manipulation of protein β-sheet structure seems critical in this regard.

1. Introduction

Many, so-called minor grains, sorghum, the millets (major species – pearl millet, foxtail millet, proso millet, finger millet, teff and fonio) and the pseudocereals (major species - amaranth, buckwheat and quinoa) continue to be important for food security and health in at-risk communities in Africa, South America and Asia. This is because of their adaptation to the harsh environmental conditions of their centres of origin and similar agro-ecological zones. However, over the past 30 years their relative, and even actual production, has declined. For example, the average annual production of sorghum in Africa over the 5-year periods 1979-1983 and 2007-2011 was 12.2 and 23.9 M tons, an increase of 93%, whereas maize production increased 119% (FAOSTAT, 2011). More starkly, annual millet production in India increased by only 22% from 9.7 to 11.8 M tons, whereas wheat production increased by 119%, from 36.8 to 80.5 M tons. World production of buckwheat actually declined by 40% over the period, from 3.5 to 2.1 M tons.

Notwithstanding this, these minor grains have some most useful quality characteristics. Notably, they are characterised by being rich in many "health-promoting" phytochemicals, which exhibit antioxidant and free-radical scavenging activity (Prybylksi et al., 1998; reviewed by Dykes and Rooney, 2006). This is perhaps because in the regions where these grains are traditionally cultivated, breeding has selected those varieties rich in phytochemicals such as phenolics, as they confer resistance to biotic stresses (Waniska et al., 1989). Sorghum and millets belong to the Andropogoneae, Eragrostideae and Paniceae tribes of the cereal grass family (Morrison and Wrigley, 2004) and thus are distantly related to the Triticeae tribe cereals (wheat, barley and rye) and the pseudocereals are dicotyledonous

plants. As such, all these minor grains are considered as "gluten-free" and suitable for persons suffering from coeliac disease and wheat-induced enteropathy, sensitivities and allergies (Arendt and Dal Bello, 2008). For these reasons, there is great interest among the public and scientists in their use for food. For example, in June 2013 Google yielded 329 000 hits for "teff recipes" and more than 28 million for "quinoa recipes". Also, this year the FAO has designated 2013 as the International Year of Quinoa (FAO, 2013).

In the early 2000s, two multi-author monographs were published on the minor grains (Belton and Taylor, 2002; Abdel-Aal and Wood, 2005). These books dealt primarily with their grain structure, chemical and nutrient composition and traditional food and beverage use. Since then, there has been huge progress in our knowledge of these grains across the spectrum of cereal science and technology (Gallagher, 2009; Arendt and Del Bello, 2009; Zannini and Arendt, 2013). This paper will review the "state-of-the-art" in the science of three topics concerning sorghum the millets and the major pseudocereals, which have become of great interest: 1. Their phenolic phytochemicals and the available scientific evidence for their health-promoting and disease-prevention action; 2. The science of biofortification to enhance their content and bioavailability of macro- and micronutrients, and 3. The potential protein functionality of such grains to enable the production of high quality bread and related dough-based-products.

The objective is to promote a deeper understanding of the unique properties and potential of these minor grains to improve human health and well-being, with the aim of increasing their cultivation productivity and utilisation, particularly in developing countries. This is crucially important as a "Nutrition Transition" from traditional grains to a "Western" high fat and high sugar diet in developing regions (Popkin, 2003), such as sub-Saharan Africa, is already

leading to dramatic increases in cardiovascular disease (Mbewu, 2009) and Type 2 diabetes (Mbanya et al., 2010).

2. Phytochemicals

2.1 Phenolic composition of sorghum, millets and pseudocereals

Phenolic compounds form a very large group of compounds containing the phenol functional group as a fundamental component. Conveniently, they may be classified based on increasing molecular weight into phenolic acids, flavonoid-type compounds and tannins. In reality, there are various classes of phenolic compounds as follows: flavonoids, phenolic acids, lignans, coumarins, phenols, phenylpropanoids, quinines, stilbenoids, and xanthones. Flavonoids, which make up the largest group among phenolics, are further subdivided into anthocyanins, flavanols including proanthocyanidins, flavonols, dihydroflavonols, flavones, isoflavonoids, flavonones, chalcones, and dihydrochalcones. Phenolic acids are further subclassified as hydroxybenzoic acids, hydroxycinnamic acids, hydroxyphenylacetic acids and hydroxyphenylpropanoic acids. Other phenolics include alkylmethoxyphenols, alkylphenols, curcuminoids, furacoumarins, hydroxybenzaldehydes, hydroxybenzoketones, hydroxycinnamaldehydes, hydroxycoumarins, hydroxyphenylpropenes, methoxyphenols, naphthoquinones, phenolic terpenes, and tyrosols. A brief discussion of the phenolic composition of sorghum, millets and pseudocereals follows.

Total phenolic content and total flavonoid content

Spectrophotometric assays such as the determination of total phenolic content using the Folin-Ciocalteu reagent and total flavonoid content using the $AlCl_3$ assay are used extensively to quantify phenolics in grains. Although these assays generally provide good information regarding trends in certain parameters, they suffer from their non-specificity. It is also difficult to make direct comparisons of values in literature as different solvents have been used to prepare extracts for analysis and also different standards have been used. Nevertheless, they offer a useful way of characterizing the grain material regarding content of phenolics.

Sorghum has a total phenolic content ranging from 3 to 43 mg/100 g (Dykes and Rooney, 2006; Ragaee et al., 2006; Sikwese and Duodu, 2007). Total phenolic and total flavonoid contents of some of the major millets have been reported by Chandrasekara and Shahidi (2010) for soluble (free and soluble esterified) and bound phenolic fractions. Typical total phenolic contents reported are 411–610 mg/100 g (finger millet), 168 mg/100 g (pearl millet) and 140 mg/100 g (proso millet) ferulic acid equivalents in the soluble phenolic fraction. For the bound phenolic fraction, total phenolic contents range from 62–74 mg/100 g (finger millet) to 178 mg/100 g (pearl millet) and 43 mg/100 g (proso millet) ferulic acid equivalents. Total flavonoid contents have been reported as 203–228 mg/100 g (finger millet), 49 mg/100 g (pearl millet) and 140 mg/100 g (proso millet) catechin equivalents in the soluble phenolic fraction. For the bound phenolic fraction, total flavonoid contents range from 10–30 mg/100 g (finger millet) to 8 mg/100 g (pearl millet) and 13 mg/100 g (proso millet) catechin equivalents.

Buckwheat has a total phenolic content ranging from 29 to 1371 mg/100 g, depending on the method of extraction, and which standard (gallic acid or ferulic acid) was used calibration

(Holasova et al., 2002; Alvarez-Jubete et al., 2010b; Gorinstein et al., 2007; Velioglu et al., 1998; Oomah et al., 1996; Cao et al., 2008; Zielinski et al., 2009). Amaranth has a total phenolic content ranging from 2 to 24 mg/100 g (Alvarez-Jubete et al., 2010b) while the total polyphenols ranges from 2 to 300 mg/100 g both on as is and dry weight basis (Pasko et al., 2009; Khandaker et al., 2008). Anthocyanin contents of 90 and 103 mg cyanidin 3-glucoside equivalents/100 g were reported (Pasko et al., 2009).

Phenolic acids and flavonoids

Various phenolic acids and flavonoids have been identified in these minor grains. The phenolic acids are mainly derivatives of benzoic acid and cinnamic acid and occur largely in bound form (especially the cinnamic acid derivatives). The flavonoids may be classified into various groups such as flavanols, flavanones, flavones, flavonones and anthocyanins. With regard to anthocyanins, the presence of 3-deoxyanthocyanins in pigmented sorghums is of considerable interest due to their potential health benefits. Table 1 gives a summary of various phenolic acids and flavonoids identified in sorghum, millets and pseudocereals.

2.2 Health-promoting properties of these minor grains

The potential health-promoting properties of grains resulting from their phytochemicals can be classified into reduction and/or prevention of oxidative stress, anti-cancer, anti-diabetic, anti-inflammatory, and cardiovascular disease prevention and anti-hypertensive.

Reduction and/or prevention of oxidative stress

Free radicals, generally in the form of reactive oxygen species or reactive nitrogen species are usual by-products of cellular redox processes in the body. It is postulated that at low

Table 1

Phenolic acids and flavonoids reported in sorghum, millets and pseudocereals

Grain sample	Phenolic compound	Reference
	Phenolic acids	
Sorghum		
	Gallic acid	Dykes and Rooney (2006); Dykes and Rooney (2007); Dicko et al., (2006)
	Protocatechuic acid	Dykes and Rooney (2006); Dykes and Rooney (2007); Dicko et al., (2006)
	p-Hydroxybenzoic acid	Dykes and Rooney (2006); Dykes and Rooney (2007); Dicko et al., (2006)
	Vanillic acid	Dykes and Rooney (2006); Dykes and Rooney (2007); Dicko et al., (2006)
	Caffeic acid	Dykes and Rooney (2006); Dykes and Rooney (2007); Dicko et al., (2006)
	p-Coumaric acid	Dykes and Rooney (2006); Dykes and Rooney (2007)
	Ferulic acid	Dykes and Rooney (2006); Dykes and Rooney (2007)
	Cinnamic acid	Dykes and Rooney (2006); Dykes and Rooney (2007)
	Gentisic acid	Dykes and Rooney (2007)
	Salicylic acid	Dykes and Rooney (2007)
	Sinapic acid	Dykes and Rooney (2007)
	Syringic acid	Dykes and Rooney (2007)
Millets		
Finger millet		
	Caffeic acid	Dykes and Rooney (2006); Dykes and Rooney (2007)
	Cinnamic acid	Dykes and Rooney (2006); Dykes and Rooney (2007)
	p-Coumaric acid	Dykes and Rooney (2007); Chandrasekara and Shahidi (2011)
	Ferulic acid	Dykes and Rooney (2006); Dykes and Rooney (2007); Chandrasekara and Shahidi (2011)
	Gentisic acid	Dykes and Rooney (2006); Dykes and Rooney (2007)
	p-Hydroxybenzoic acid	Dykes and Rooney (2006); Dykes and Rooney (2007)
	Protocatechuic acid	Dykes and Rooney (2006); Dykes and Rooney (2007)
	Sinapic acid	Dykes and Rooney (2007)
	Syringic acid	Dykes and Rooney (2006); Dykes and Rooney (2007)
	Vanillic acid	Dykes and Rooney (2006); Dykes and Rooney (2007)
Kodo millet		
	Ferulic acid	Chandrasekara and Shahidi (2011)
	p-Coumaric acid	Chandrasekara and Shahidi (2011)
Little millet		
	Ferulic acid	Chandrasekara and Shahidi (2011)

	p-Coumaric acid	Chandrasekara and Shahidi (2011)
Proso millet		
	Ferulic acid	Chandrasekara and Shahidi (2011)
	p-Coumaric acid	Chandrasekara and Shahidi (2011)
Foxtail millet		
	Caffeic acid	Dykes and Rooney (2006); Dykes and Rooney (2007)
	Cinnamic acid	Dykes and Rooney (2006); Dykes and Rooney (2007)
	p-Coumaric acid	Dykes and Rooney (2007); Chandrasekara and Shahidi (2011)
	Ferulic acid	Dykes and Rooney (2006); Dykes and Rooney (2007); Chandrasekara and Shahidi (2011)
	Gentisic acid	Dykes and Rooney (2006); Dykes and Rooney (2007)
	p-Hydroxybenzoic acid	Dykes and Rooney (2006); Dykes and Rooney (2007)
	Protocatechuic acid	Dykes and Rooney (2006); Dykes and Rooney (2007)
	Syringic acid	Dykes and Rooney (2006); Dykes and Rooney (2007)
	Vanillic acid	Dykes and Rooney (2006); Dykes and Rooney (2007)
Pearl millet		
	Caffeic acid	Dykes and Rooney (2006); Dykes and Rooney (2007)
	Cinnamic acid	Dykes and Rooney (2006); Dykes and Rooney (2007)
	p-Coumaric acid	Dykes and Rooney (2007); Chandrasekara and Shahidi (2011)
	Ferulic acid	Dykes and Rooney (2006); Dykes and Rooney (2007); Chandrasekara and Shahidi (2011)
	Gentisic acid	Dykes and Rooney (2006); Dykes and Rooney (2007)
	p-Hydroxybenzoic acid	Dykes and Rooney (2006); Dykes and Rooney (2007)
	Protocatechuic acid	Dykes and Rooney (2006); Dykes and Rooney (2007)
	Sinapic acid	Dykes and Rooney (2007)
	Syringic acid	Dykes and Rooney (2006); Dykes and Rooney (2007)
	Vanillic acid	Dykes and Rooney (2006); Dykes and Rooney (2007)
Amaranth		
	Protocatechuic acid	Alvarez-Jubete et al. (2010b)
	p-Hydroxybenzoic acid	Pasko et al. (2008); Barba de la Rosa et al. (2009); Shobana et al. (2009)
	Syringic acid	Barba de la Rosa et al. (2009)
	Gallic acid	Pasko et al (2008)
	p-Coumaric acid	Pasko et al. (2008)
	Vanillic acid	Pasko et al. (2008)
Buckwheat		
	Ferulic acid	Przybylski et al. (1998); Guo et al. (2011)
	p-Coumaric acid	Gallardo et al. (2006); Guo et al. (2011)

	o-Coumaric acid	Przybylski et al. (1998)
	p-Hydroxybenzoic acid	Gallardo et al. (2006); Guo et al. (2011)
	Sinapic acid	Gallardo et al. (2006)
	Caffeic acid	Przybylski et al. (1998); Guo et al. (2011)
	Vanillic acid	Gallardo et al. (2006)
	Gallic acid	Przybylski et al. (1998); Guo et al. (2011)
Quinoa		
	Caffeic acid	Pasko et al. (2008); Repo-Carrasco-Valencia et al. (2010)
	Ferulic acid	Okarter (2012)
	p-Coumaric acid	Okarter (2012)
	p-Hydroxybenzoic acid	Pasko et al. (2008); Okarter (2012)
	Vanillic acid	Pasko et al. (2008); Alvarez-Jubete et al. (2010); Okarter (2012)
	Gallic acid	Pasko et al. (2008)
	Cinnamic acid	Pasko et al. (2008)
	Protocatechuic acid	Alvarez-Jubete et al. (2010b)
	Flavonoids	
Sorghum		
	Anthocyanins	Kaluza and McGrath (1980); Awika et al. (2004); Dicko et al. (2005)
	Apigeninidin and derivatives	Dykes and Rooney (2007)
	Luteolinidin and derivatives	Dykes and Rooney (2007)
	Apigenin	Dykes and Rooney (2007)
	Luteolin	Dykes and Rooney (2007)
	Eriodictyol and derivatives	Dykes and Rooney (2007)
	Kaempferol	Dykes and Rooney (2007)
	Taxifolin	Dykes and Rooney (2007)
	Apiforol	Dykes and Rooney (2007)
	Luteoforol	Dykes and Rooney (2007)
	Catechin	Dykes and Rooney (2007)
	Procyanidin	Dykes and Rooney (2007)
Millets		
Pearl millet		
	Vitexin	Dykes and Rooney (2007)
	Glucosylorientin	Dykes and Rooney (2007)
	Glucosylvitexin	Dykes and Rooney (2007)
Fonio		
	Apigenin	Dykes and Rooney (2007)

	Luteolin	Dykes and Rooney (2007)
Japanese barnyard		
	Luteolin	Dykes and Rooney (2007)
	Tricin	Dykes and Rooney (2007)
Amaranth		
	Rutin	Barba de la Rosa et al. (2009); Kalinova and Dadakova (2009)
	Isoquercetin	Barba de la Rosa et al. (2009)
	Quercetin	Kalinova and Dadakova (2009)
	Nicotiflorin	Khandaker et al. (2008)
	Anthocyanins	Pasko et al. (2009)
Buckwheat		
	Quercetin	Przybylski et al. (1998); Quettier-Deleu et al. (2000); Morishita et al. (2007); Cao et al. (2008); Guo et al. (2011)
	Quercetin-3-O-rutinoside	Pasko et al. (2008)
	Rutin	Kreft et al. (1999); Quettier-Deleu et al. (2000); Jiang et al. (2007); Morishita et al. (2007); Cao et al. (2008)
	Isovitexin	Dietrych-Szostak and Oleszek (1999); Zielinski et al. (2009)
	Vitexin	Zielinski et al. (2009)
	Homoorientin	Zielinski et al. (2009)
	Orientin	Dietrych-Szostak and Oleszek (1999); Zielinski et al. (2009)
	Isoorientin	Dietrych-Szostak and Oleszek (1999)
	Hyperoside	Quettier-Deleu et al. (2000)
	Epicatechin	Quettier-Deleu et al. (2000)
	Epicatechin-3-O-gallate	Quettier-Deleu et al. (2000)
Quinoa		
	Myricetin	Repo-Carrasco-Valencia et al. (2010)
	Quercetin	Alvarez-Jubete et al. (2010); Repo-Carrasco-Valencia et al. (2010)
	Kaempferol	Alvarez-Jubete et al. (2010); Repo-Carrasco-Valencia et al. (2010)
	Isohamnetin	Repo-Carrasco-Valencia et al. (2010)
	Rutin	Pasko et al. (2008)
	Orientin	Pasko et al. (2008)
	Vitexin	Pasko et al. (2008)
	Morin	Pasko et al. (2008)
	Hesperidin	Pasko et al. (2008)
	Neohesperidin	Pasko et al. (2008)

concentrations, these have beneficial effects on cellular responses and immune function (Pham-Huy et al., 2008). However, at high concentrations they cause the condition known as oxidative stress which is harmful to cell structures. In simple terms, oxidative stress has been defined as the condition whereby the balance between formation and removal of free radicals is shifted towards formation of more free radicals (Shinde et al., 2012). The generally held hypothesis is that oxidative stress plays a major role in the development of chronic and degenerative diseases such as cancer, cardiovascular disease and diabetes (Pham-Huy et al., 2008; Fearon and Faux, 2009).

The human body has its own endogenous antioxidant system designed to counteract oxidative stress and this is supported by exogenously supplied dietary antioxidants (Pham-Huy et al., 2008). In this regard, phytochemicals in grains such as phenolic compounds are believed to potentially play a role in protecting the body against oxidative stress and its effects due to their well-known antioxidant properties. The in vitro antioxidant capacity or activity of extracts from grains is well documented and is used as an indicator of the potential ability of dietary antioxidants to counteract oxidative stress. This has been determined mostly as in vitro free radical scavenging ability although other assays have been used such as inhibition of lipid peroxidation and metal ion chelating capacity. Table 2 provides a summary of some antioxidant capacities of sorghum, millets and pseudocereals.

Efforts to demonstrate the potential of grain antioxidants to counteract oxidative stress have been extended to in vivo studies, mostly animals. In the main, this involves monitoring the activities of antioxidant enzymes or antioxidant molecules within the experimental animal on feeding with the grain of interest (Hegde et al., 2005; Pasko et al., 2010a; Moraes et al., 2012; Lee et al., 2013).

Table 2

In vitro antioxidant activities of sorghum, millets and pseudocereals

Sample	In vitro antioxidant activity	Reference
	ABTS* radical scavenging	
Sorghum		
Black sorghum bran (acidified methanol extracts)	190 – 400 µmol Trolox equivalents/g	Awika et al. (2004)
Black sorghum grain (acidified methanol extracts)	52 – 112 µmol Trolox equivalents/g	Awika et al. (2004)
Red sorghum grains (ungerminated and germinated)	30 – 80 µmol Trolox equivalents/g	Dicko et al. (2005)
White sorghum grains (ungerminated and germinated)	16 – 62 µmol Trolox equivalents/g	Dicko et al. (2005)
Whole grain sorghum (aqueous methanol extract)	51.7 µmol ABTS scavenged/g	Ragaee et al. (2006)
Non-tannin whole grain (aqueous acetone extracts)	63.9 – 78.9 µmol Trolox equivalents/g	Awika et al (2009)
Tannin whole grain (aqueous acetone extracts)	61.6 – 125 µmol Trolox equivalents/g	Awika et al. (2009)
Millets		
Whole grain pearl millet (aqueous methanol extract)	21.4 µmol ABTS scavenged/g	Ragaee et al. (2006)
Kodo millet (soluble phenolic extract)	41.68 µmol Trolox equivalents/g	Chandrasekara and Shahidi (2010)
Kodo millet (bound phenolic extract)	86.13 µmol Trolox equivalents/g	Chandrasekara and Shahidi (2010)
Finger millet (soluble phenolic extract)	6.29 – 12.37 µmol Trolox equivalents/g	Chandrasekara and Shahidi (2010)
Finger millet (bound phenolic extract	5.03 – 6.77 µmol Trolox equivalents/g	Chandrasekara and Shahidi (2010)
Foxtail millet (soluble phenolic extract)	11.14 µmol Trolox equivalents/g	Chandrasekara and Shahidi (2010)
Foxtail millet (bound phenolic extract)	40.61 µmol Trolox equivalents/g	Chandrasekara and Shahidi (2010)
Proso millet (soluble phenolic extract)	6.73 µmol Trolox equivalents/g	Chandrasekara and Shahidi (2010)
Proso millet (bound phenolic extract)	11.14 µmol Trolox equivalents/g	Chandrasekara and Shahidi (2010)
Little millet (soluble phenolic extract)	3.70 µmol Trolox equivalents/g	Chandrasekara and Shahidi (2010)
Little millet (bound phenolic extract)	18.34 µmol Trolox equivalents/g	Chandrasekara and Shahidi (2010)
Pearl millet (soluble phenolic extract)	4.15 µmol Trolox equivalents/g	Chandrasekara and Shahidi (2010)
Pearl millet (bound phenolic extract)	6.77 µmol Trolox equivalents/g	Chandrasekara and Shahidi (2010)
Amaranth		
Seeds (acidified methanol/70% acetone extracts)	11.42 – 12.71 mmol Trolox equivalents/kg	Pasko et al. (2009)
Amaranth grain (acidified methanol extract)	3.7 µmol Trolox equivalents/g	Chirinos et al. (2013)
Quinoa		

Seeds (acidified methanol/70% acetone extracts)	27.19 mmol Trolox equivalents/kg	Pasko et al. (2009)
Quinoa grain (acidified methanol extract)	8.3 µmol Trolox equivalents/g	Chirinos et al. (2013)
Buckwheat		
Whole grain (water extract, freeze-dried)	0.138 µmol Trolox equivalents/mg	Zielinski and Kozlowska (2000)
Whole grain (80% methanol extract, freeze-dried)	0.587 µmol Trolox equivalents/mg	Zielinski and Kozlowska (2000)
Dehulled grain (80% methanol extract, freeze-dried)	0.548 µmol Trolox equivalents/mg	Zielinski and Kozlowska (2000)
Hulls (80% methanol extract, freeze-dried)	1.175 µmol Trolox equivalents/mg	Zielinski and Kozlowska (2000)
Endosperm / embryo (80% methanol extract, freeze-dried)	0.598 µmol Trolox equivalents/mg	Zielinski and Kozlowska (2000)
Buckwheat bran (80% methanol extract)	24.24 µmol Trolox equivalents/g	Zdunczyk et al. (2006)
Buckwheat hulls (80% methanol extract)	26.15 µmol Trolox equivalents/g	Zdunczyk et al. (2006)
Whole buckwheat (80% methanol extract)	42.24 µmol Trolox equivalents/g	Zielinska et al. (2007)
Hydrothermally treated whole buckwheat (80% methanol extract)	12.59 µmol Trolox equivalents/g	Zielinska et al. (2007)
Roasted buckwheat groats (80% methanol extracts)	9.25 µmol Trolox equivalents/g	Zielinska et al. (2007)
Buckwheat hulls (80% methanol extracts	13.53 µmol Trolox equivalents/g	Zielinska et al. (2007)
Whole buckwheat seeds	28.60 µmol Trolox equivalents/g	Zielinski et al. (2009)
Roasted whole buckwheat seeds	25.57 µmol Trolox equivalents/g	Zielinski et al. (2009)
Buckwheat groats	31.28 µmol Trolox equivalents/g	Zielinski et al. (2009)
Roasted buckwheat groats	25.12 µmol Trolox equivalents/g	Zielinski et al. (2009)
	DPPH radical scavenging**	
Sorghum		
Whole grain sorghum (aqueous methanol extract)	195.8 µmol DPPH scavenged/g	Ragaee et al. (2006)
Non-tannin whole grain (aqueous acetone extracts)	15.3 – 22.2 µmol Trolox equivalents/g	Awika et al. (2009)
Tannin whole grain (aqueous acetone extracts)	17.7 – 44.7 µmol Trolox equivalents/g	Awika et al (2009)
Millets		
Whole grain pearl millet (aqueous methanol extract)	23.83 µmol DPPH scavenged/g	Ragaee et al. (2006
Kodo millet (soluble phenolic extract)	25.69 µmol ferulic acid equivalents/g	Chandrasekara and Shahidi (2011)
Kodo millet (bound phenolic extract)	17.38 µmol ferulic acid equivalents/g	Chandrasekara and Shahidi (2011)
Finger millet (soluble phenolic extract)	29.43 – 54.40 µmol ferulic acid equivalents/g	Chandrasekara and Shahidi (2011)
Finger millet (bound phenolic extract	5.84 – 6.93 µmol ferulic acid equivalents/g	Chandrasekara and Shahidi (2011)
Foxtail millet (soluble phenolic extract)	4.54 µmol ferulic acid equivalents/g	Chandrasekara and Shahidi (2011)

Foxtail millet (bound phenolic extract)	5.36 µmol ferulic acid equivalents/g	Chandrasekara and Shahidi (2011)
Proso millet (soluble phenolic extract)	3.72 µmol ferulic acid equivalents/g	Chandrasekara and Shahidi (2011)
Proso millet (bound phenolic extract)	2.77 µmol ferulic acid equivalents/g	Chandrasekara and Shahidi (2011)
Little millet (soluble phenolic extract)	7.59 µmol ferulic acid equivalents/g	Chandrasekara and Shahidi (2011)
Little millet (bound phenolic extract)	4.19 µmol ferulic acid equivalents/g	Chandrasekara and Shahidi (2011)
Pearl millet (soluble phenolic extract)	9.95 µmol ferulic acid equivalents/g	Chandrasekara and Shahidi (2011)
Pearl millet (bound phenolic extract)	7.14 µmol ferulic acid equivalents/g	Chandrasekara and Shahidi (2011)
Amaranth		
Whole grain (ethanolic extracts)	79.4 – 85.6% DPPH scavenged	Nsimba et al. (2008)
Seeds (acidified methanol/70% acetone extracts)	3.15 – 4.42 mmol Trolox equivalents/kg	Pasko et al. (2009)
Amaranth grain (acidified methanol extract)	1.2 µmol Trolox equivalents/g	Chirinos et al. (2013)
Quinoa		
Whole grain (ethanolic extract)	59.2 – 72.1% DPPH scavenged	Nsimba et al. (2008)
Seeds (acidified methanol/70% acetone extracts)	38.84 mmol Trolox equivalents/kg	Pasko et al. (2009)
Sweet quinoa, raw seeds (aqueous methanol extract)	28.7 µmol Trolox equivalents/10 g	Dini et al. (2010)
Sweet quinoa, boiled seeds (aqueous methanol extract)	19.9 µmol Trolox equivalents/10 g	Dini et al. (2010)
Bitter quinoa, raw seeds (aqueous methanol extract)	67.1 µmol Trolox equivalents/10 g	Dini et al. (2010)
Bitter quinoa, boiled seeds (aqueous methanol extract)	35.7 µmol Trolox equivalents/10 g	Dini et al. (2010)
Whole grain quinoa (aqueous methanol extracts	502 – 950 µmol Trolox equivalents/100 g	Hirose et al. (2010)
Quinoa grain (acidified methanol extract)	5.3 µmol Trolox equivalents/g	Chirinos et al. (2013)
Buckwheat		
White buckwheat flour (methanolic extract)	2.14 µmol Trolox equivalents/g	Sensoy et al. (2006)
Whole buckwheat flour (methanolic extract)	2.13 µmol Trolox equivalents/g	Sensoy et al. (2006)
Extruded dark buckwheat flour (methanolic extract)	2.12 µmol Trolox equivalents/g	Sensoy et al. (2006)
Roasted dark buckwheat flour (methanolic extract)	1.85 µmol Trolox equivalents/g	Sensoy et al. (2006)
Buckwheat flour (water extract)	5897 µmol Trolox equivalents/100 g	Gallardo et al. (2006)
Buckwheat flour (methanol extract)	1435 µmol Trolox equivalents/100 g	Gallardo et al. (2006)
Whole buckwheat (80% methanol extract)	27.20 µmol Trolox equivalents/g	Zielinska et al. (2007)
Hydrothermally treated whole buckwheat (80% methanol extract)	8.22 µmol Trolox equivalents/g	Zielinska et al. (2007)

Roasted buckwheat groats (80% methanol extracts)	5.48 µmol Trolox equivalents/g	Zielinska et al. (2007)
Buckwheat hulls (80% methanol extracts	13.16 µmol Trolox equivalents/g	Zielinska et al. (2007)
Common buckwheat flour (80% ethanol extracts)	15.3 – 16.4 µmol Trolox equivalents/g	Morishita et al. (2007)
Tartary buckwheat flour (80% ethanol extracts	52.9 – 57.4 µmol Trolox equivalents/g	Morishita et al. (2007)
Normal buckwheat sprouts (6-10 days, with hulls)	1.5 – 1.8 µmol Trolox equivalents/g	Kim et al. (2008)
Normal buckwheat sprouts (6-10 days, without hulls)	1.5 – 1.9 µmol Trolox equivalents/g	Kim et al. (2008)
Tartary buckwheat sprouts (6-10 days)	1.5 – 1.7 µmol Trolox equivalents/g	Kim et al. (2008)
	Oxygen radical absorbance capacity (ORAC)	
Sorghum		
Non-tannin whole grain (aqueous acetone extracts)	81.6 – 126 µmol Trolox equivalents/g	Awika et al. (2009)
Tannin whole grain (aqueous acetone extracts)	72.4 – 236 µmol Trolox equivalents/g	Awika et al. (2009)
Black sorghum bran (80% methanol extract)	3.7 µmol Trolox equivalents/mg	González-Montilla et al. (2012)
Millets		
Kodo millet (soluble phenolic extract)	74.25 ferulic acid equivalents/g	Chandrasekara and Shahidi (2011)
Kodo millet (bound phenolic extract)	606.88 ferulic acid equivalents/g	Chandrasekara and Shahidi (2011)
Finger millet (soluble phenolic extract)	71.90 – 115.05 ferulic acid equivalents/g	Chandrasekara and Shahidi (2011)
Finger millet (bound phenolic extract	44.32 – 85.24 ferulic acid equivalents/g	Chandrasekara and Shahidi (2011)
Foxtail millet (soluble phenolic extract)	42.34 ferulic acid equivalents/g	Chandrasekara and Shahidi (2011)
Foxtail millet (bound phenolic extract)	202.64 ferulic acid equivalents/g	Chandrasekara and Shahidi (2011)
Proso millet (soluble phenolic extract)	23.93 ferulic acid equivalents/g	Chandrasekara and Shahidi (2011)
Proso millet (bound phenolic extract)	90.86 ferulic acid equivalents/g	Chandrasekara and Shahidi (2011)
Little millet (soluble phenolic extract)	64.46 ferulic acid equivalents/g	Chandrasekara and Shahidi (2011)
Little millet (bound phenolic extract)	172.39 ferulic acid equivalents/g	Chandrasekara and Shahidi (2011)
Pearl millet (soluble phenolic extract)	41.73 ferulic acid equivalents/g	Chandrasekara and Shahidi (2011)
Pearl millet (bound phenolic extract)	125.44 ferulic acid equivalents/g	Chandrasekara and Shahidi (2011)
Amaranth		
Amaranth grain (acidified methanol extract)	6.5 µmol Trolox equivalents/g	Chirinos et al. (2013)
Quinoa		
Quinoa grain (acidified methanol extract)	15.9 µmol Trolox equivalents/g	Chirinos et al. (2013)
Buckwheat		
Buckwheat flour (water extract)	3351 µmol Trolox equivalents/100 g	Gallardo et al. (2006)

Buckwheat flour (methanol extract)	705 µmol Trolox equivalents/100 g	Gallardo et al. (2006)
Whole buckwheat seeds	130.31 µmol Trolox equivalents/g (lipophilic + hydrophilic)	Zielinski et al. (2009)
Roasted whole buckwheat seeds	72.21 µmol Trolox equivalents/g (lipophilic + hydrophilic)	Zielinski et al. (2009)
Buckwheat groats	99.70 µmol Trolox equivalents/g (lipophilic + hydrophilic)	Zielinski et al. (2009)
Roasted buckwheat groats	46.09 µmol Trolox equivalents/g (lipophilic + hydrophilic)	Zielinski et al. (2009)
	Ferric reducing antioxidant power (FRAP)	
Amaranth		
Seeds (acidified methanol/70% acetone extracts)	3.37 – 3.73 mg Fe^{2+}/kg	Pasko et al. (2008)
Sprouts (acidified methanol/70% acetone extracts)	61.1 – 248.1 mg Fe^{2+}/kg	Pasko et al. (2008)
Whole grain (ethanolic extracts)	7.3 – 7.6 mmol/L equivalent to 1 mM $FeSO_4$	Nsimba et al. (2008)
Quinoa		
Seeds (acidified methanol/70% acetone extracts)	4.97 mg Fe^{2+}/kg	Pasko et al. (2008)
Sprouts (acidified methanol/70% acetone extracts)	31.4 – 77.4 mg Fe^{2+}/kg	Pasko et al. (2008)
Whole grain (ethanolic extracts)	7.5 – 8.2 mmol/L equivalent to 1 mM $FeSO_4$	Nsimba et al. (2008)
Sweet quinoa, raw seeds (aqueous methanol extract)	25.6 µmol Trolox equivalents/10 g	Dini et al. (2010)
Sweet quinoa, boiled seeds (aqueous methanol extract)	12.7 µmol Trolox equivalents/10 g	Dini et al. (2010)
Bitter quinoa, raw seeds (aqueous methanol extract)	87.3 µmol Trolox equivalents/10 g	Dini et al. (2010)
Bitter quinoa, boiled seeds (aqueous methanol extract)	47.7 µmol Trolox equivalents/10 g	Dini et al. (2010)

*ABTS – 2,2'-azinobis (3-ethyl-benzothiazoline-6-sulphonic acid)

**DPPH – 1,1-diphenyl-2-picrylhydrazyl

Pasko et al. (2010a) fed male Wistar rats with fructose-containing diets (to induce oxidative stress) and then with quinoa-supplemented diets. Fructose administration caused oxidative stress by bringing about an increase in plasma malondialdehyde concentration and non-significant changes in enzymatic antioxidant potential in plasma and most tissues. Quinoa supplementation maintained normal activities of some enzymes. It led to decrease in plasma malondialdehyde concentration and increased activities of the antioxidant enzymes catalase and glutathione peroxidase in the pancreas . Overall, quinoa supplementation reduced lipid peroxidation and enhanced antioxidant capacity of blood (plasma), heart, kidney, testes, lung and pancreas.

It is generally accepted therefore that oxidative stress is a precursor for the development of various chronic and degenerative diseases. A brief discussion of some of these diseases and the potential role of phytochemicals in these minor grains in their prevention follows. Table 3 shows some examples of reported health-promoting properties of these minor grains.

Anti-cancer properties

Various reportsprovide some evidence of potential anti-cancer properties of sorghum, millets and pseudocereals. Anti-cancer properties of these grains have been demonstrated in mostly in vitro assays through assays such as their effects on oxidative stress (free radical scavenging), inhibition of DNA damage, antiproliferative effects on cancer cell lines and Phase II enzyme induction.

Extracts from sorghum have been reported to exhibit Phase II enzyme inducing properties and cancer cell antiproliferative properties depending on sorghum type (Awika et al., 2009). An extract from black (non-tannin) sorghum, which is high in 3-deoxyanthocyanins, was the

Table 3

Some health-promoting properties of sorghum, millets and pseudocereals

Health-promoting property	Sample	Methodology	Key findings	Reference
Anti-cancer properties				
	Acidified aqueous acetone extracts of tannin and non-tannin sorghums	Quinone oxidoreductase inducer activity and antiproliferative potential against esophageal (OE33) and colon (HT-29) carcinoma cells	Extracts from black sorghum had highest phase II enzyme inductor activity compared to white sorghum. Extracts from tannin sorghums were most potent at preventing proliferation of cancer cells	Awika et al. (2009)
	Acidified aqueous acetone extracts of black, red and white sorghum	Quinone oxidoreductase inducer activity and antiproliferative potential against colon (HT-29) carcinoma cells	Black sorghum extracts with high levels of methoxylated 3-deoxyanthocyanins induced quinone reductase activity strongly. All extracts inhibited proliferation of HT-29 carcinoma cells	Yang et al. (2009)
	Methanolic extracts from black sorghum bran	Quinone oxidoreductase inducer activity in murine hepatoma cells (Hepa 1c1c7)	Extracts showed significant quinone reductase induction without significant cytotoxicity. A derivative of 7-methoxyapigeninidin from the extracts was identified as having significant quinone reductase inducer activity.	González-Montilla et al. (2012)
	Methanolic anthocyanin extracts from red sorghum bran	Determination of inhibitory effect on deoxyribose degradation induced by hydroxyl radicals	Extracts showed significant reduction in degradation of DNA and increased native form of DNA	Suganyadevi et al. (2012)

	Purified anthocyanin extract (acidified methanol) from red sorghum bran	Antiproliferative activity against human breast cancer cell line (MCF-7)	The extract induced apoptosis of MCF-7 cells by stimulation of P53 gene and down regulation of Bcl-2 gene.	Suganyadevi et al. (2013)
Anti-diabetic properties				
	Extracts from 2 day buckwheat sprouts and 10 day seedlings	Inhibitory activity against α-amylase and α-glucosidase	Increases in inhibitory activity against α-amylase and α-glucosidase correlated with increases in total phenolic content and antioxidant activity	Randhir et al. (2008)
	Ethanolic extracts from sorghum, foxtail millet and proso millet	Inhibitory activity against α-amylase and α-glucosidase	Sorghum extracts had higher inhibitory activity against α-glucosidase compared to acarbose. Sorghum extracts also inhibited pancreatic and salivary α-amylase. Foxtail and proso millet extracts showed no detectable enzyme inhibitory activity	Kim et al. (2011)
	Methanolic extracts from raw and processed (soaking/cooking and roasting) finger millet and amaranth grains	Inhibitory activity against α-amylase and α-glucosidase	All extracts inhibited α-amylase and α-glucosidase. Inhibition against α-glucosidase was relatively higher than against α-amylase	Kunyanga et al. (2012)
	Kodo millet and finger millet-enriched diets	In vivo study of the effect of millet-enriched diets on blood glucose and oxidative stress in alloxan-induced rats	Millet-enriched diets decreased blood glucose and reduced oxidative stress in the rats. Normal levels of enzymatic and non-enzymatic antioxidants were restored in diabetic rats when fed the millet-enriched	Hegde et al. (2005)

			diets	
	Ethanolic extracts from sorghum	In vivo study of effect of oral administration of sorghum extract on hepatic gluconeogenesis and glucose uptake by muscle in streptozotocin-induced diabetic rats	Oral administration of sorghum extract significantly reduced blood glucose concentration by inhibition of hepatic gluconeogenesis	Kim and Park (2012)
Anti-inflammatory properties				
	Ethanolic extracts from sorghum bran	Monitoring release of pro-inflammatory cytokines (IL-1β and TNF-α in human peripheral blood mononuclear cells. Effect on mouse ear inflammation was also determined	Ethanolic extract of black sorghum significantly inhibited secretion of IL-1β and TNF-α. Extracts from black and sumac sorghum bran significantly reduced edema in inflamed mouse ears	Burdette et al. (2010)
	Experimental diets enriched with tannin and non-tannin sorghum flour	In vivo study to monitor markers of inflammation and oxidative stress in rats fed hyperlipidic sorghum-enriched diets	There was lower expression of TNF-α in epididymal adipose of adult male rats. There was also lower levels of thiobarbituric acid reactive substances (TBARS) in livers of rats fed the diet enriched with tannin sorghum flour.	Moraes et al. (2012)
	Ethanolic extracts from tartary buckwheat grains	In vivo study of effect of ethanolic extracts on levels of liver toxicity indicators, inflammatory factors and antioxidant enzymes in mice and rats	Extracts inhibited increases in liver toxicity indicators, reduced inflammatory factors in the liver and increased the activity of antioxidant enzymes. The observed effects were possibily due to content of phenolic	Lee et al. (2013)

			compounds such as rutin and quercetin	
Cardiovascular disease prevention and anti-hypertensive properties				
	Methanolic extracts from three buckwheat species	Inhibition of LDL oxidation	Extracts exhibited inhibition of LDL oxidation in a dose-dependent manner. There was high positive correlation between inhibition of LDL oxidation and contents of rutin and total flavonoids	Jiang et al. (2007)
	Extracts from 2 day buckwheat sprouts and 10 day seedlings	Angiotensin converting enzyme (ACE) inhibitory activity	Extracts exhibited significant inhibition of ACE	Randhir et al. (2008)
	Meals from buckwheat seeds and sprouts	In vivo work to determine the effect of administering the buckwheat seeds and sprouts in meals on LDL and HDL cholesterol in hamsters	There was significant cholesterol lowering effects with suppression of LDL cholesterol and elevation of HDL cholesterol	Lin et al. (2008)
	Hydroalcoholic extract from quinoa seed coats	Determination of effect on peroxidation of rat liver microsomes and oxidative stress	Quinoa extract inhibited peroxidation of rat liver microsomes and prevented loss of microsomal thiol content	Letelier et al. (2011)
	Neo-fermented buckwheat sprouts (produced from lactic acid-fermented buckwheat sprouts)	In vivo work to determine blood pressure lowering effects in hypertensive rats	The fermented buckwheat sprouts decreased systolic and diastolic blood pressure, decreased ACE enzyme activity in various organs and produced a significant vasorelaxatory effect	Nakamura et al. (2013)

most potent inducer of quinone oxidoreductase, a Phase II detoxifying enzyme. White sorghum extract was, comparatively, a moderately strong inducer. Although tannin sorghum extracts were non inducers of quinone oxidoreductase, they had the strongest antiproliferative activity against human oesophageal and colon cancer cells.

Similar findings of the Phase II enzyme inducer activity of sorghum 3-deoxyanthocyanins were reported by Yang et al. (2009). A crude extract from black sorghum containing high levels of methoxylated 3-deoxyanthocyanin was a strong inducer of quinone oxidoreductase compared to red or white sorghum extracts, which contained little or no methoxylated 3-deoxyanthocyanin. Extracts from all sorghum types inhibited proliferation of HT-29 human colon cancer cells. It was apparent from this research that methoxylation of 3-deoxyanthocyanins appears to be important for induction of quinone oxidoreductase activity, which also enhances inhibition of tumour cell growth.

González-Montilla et al. (2012) reported that extracts from black sorghum grain were able to induce quinine reductase activity in murine hepatoma cells (Hepa 1c1c7) without exerting significant cytotoxic effects. A conjugated form of 7-methoxyapigeninidin was identified in the extracts as having an especially significant quinone reductase induction activity. This appears to confirm the observation by Yang et al. (2009) about the apparent need for some form of conjugation (methoxylation) of 3-deoxyanthocyanins to bring about induction of quinone reductase activity.

A recent report (Suganyadevi et al., 2013) indicates that extracts from red sorghum bran were able to induce apoptosis of MCF-7 human breast cancer cells and prevent their proliferation.

The authors presented evidence indicating that the apoptotic effect was mediated by stimulation of the P53 gene and down regulation of the Bcl-2 gene.

Cao et al. (2008) reported on effects of extracts from common and tartary buckwheat on oxidative DNA damage. All ethanolic extracts inhibited non-site-specific hydroxyl radical-mediated DNA damage at a concentration of 0.2 mg/ml. Tartary buckwheat extracts exhibited higher inhibition of oxidative DNA damage than extracts from common buckwheat, probably due to higher rutin, quercetin and total phenolic contents of the former. Inhibition of hydroxyl radical-induced DNA damage by an anthocyanin extract from red sorghum bran has also been reported (Suganyadevi et al., 2012).

Anti-diabetic properties

Diabetes is associated with various conditions including oxidative stress and impaired insulin secretion and insulin resistance due to malfunctioning of β cells of the pancreas (Sancho and Pastore, 2012). Due to their antioxidant properties, phenolic compounds may reduce oxidative stress conditions and also protect pancreatic β cells. Another widely used way of demonstrating anti-diabetic effects of phytochemicals is by determining their inhibitory effects against starch-degrading enzymes such as α-amylase and α-glucosidase.

Extracts from various minor grains have been shown to possess inhibitory effects against α-amylase and α-glucosidase. Extracts from 2 day buckwheat sprouts and 10 day seedlings had increased α-amylase and α-glucosidase inhibitory activities, which correlated with increases in total phenolics and antioxidant activity on thermal processing (Randhir et al., 2008). Ethanolic extracts from sorghum showed higher inhibitory effects against α-glucosidase than acarbose (a reference pharmaceutical) (Kim et al., 2011). The sorghum extracts also strongly

inhibited pancreatic and salivary α-amylase. Methanolic extracts from raw and processed finger millet and amaranth grains showed inhibition against α-amylase and α-glucosidase activities (Kunyanga et al., 2012). Alpha-amylase inhibition was in the range 14–26% for finger millet extracts and 21–35% for amaranth extracts. Alpha-glucosidase inhibition was 63–91% for finger millet extracts and 14–40% for amaranth grain extracts.

Some in vivo studies using experimental animals to investigate the anti-diabetic effects of grains has also been reported. Such studies have mainly involved determination of levels of blood glucose and antioxidant status after administering the grain in a diet to the experimental animal. Hegde et al. (2005) reported that diets containing whole grain meal of various millet species could protect against hyperglycaemia and alloxan-induced oxidative stress in rats. Administration of a kodo millet-enriched diet produced greater reductions in blood glucose (42%) and cholesterol (27%) in the rats than a finger millet-enriched diet (36% and 13%, respectively). There was restoration of levels of enzymatic (superoxide dismutase, catalase, glutathione peroxidise and glutathione reductase) and non-enzymatic (glutathione, vitamins E and C) antioxidants in diabetic rats fed the millet-enriched diets. This is an indication of reduction of oxidative stress upon administration of the millet diets.

Sorghum phenolic extracts have been shown to exert hypoglycaemic effects in streptozotocin-induced diabetic rats for 14 days by reducing serum glucose (Chung et al., 2011). Similar results were reported by Kim and Park (2012) who reported reduction in the concentration of triglycerides, total and LDL-cholesterol and blood glucose in streptozotocin-induced diabetic rats. The reduction in blood glucose was shown to be due to inhibition of hepatic gluconeogenesis.

Anti-inflammatory properties

Inflammation may be described as an immune response to cellular or tissue injury or infection by pathogens (Issa et al., 2006). The condition of inflammation itself is not considered a disease. However, if left unchecked as in chronic inflammation, there can be exacerbated tissue damage and modulation of various cell signalling pathways (Serhan and Savill, 2005). These can trigger the onset of various lifestyle diseases. The process of inflammation consists of a wide and complex range of cellular and molecular pathways and reactions involving a host of enzymes (Issa et al., 2006). These enzymes include cyclooxygenase, lipoxygenase, phospholipase A2 and nitric oxide synthase. Cytokines such as IL-1β and TNF-α (Tumour Necrosis Factor-α) and nitric oxide are important products of the reactions leading to inflammation. Phytochemicals can modulate inflammatory processes by inhibition of the pro-inflammatory enzymes (Issa et al., 2006), which influences production of the cytokines and nitric oxide. The anti-inflammatory properties of dietary phytochemicals may be determined by their inhibitory activity against the pro-inflammatory enzymes and by monitoring production of the pro-inflammatory cytokines. Simple in vitro nitric oxide radical scavenging capacity could also be used as an indicator of anti-inflammatory properties.

Burdette et al. (2010) have reported in vitro and in vivo anti-inflammatory activities of sorghum bran. Ethanolic extract of black sorghum bran significantly inhibited the secretion of the pro-inflammatory cytokines IL-1β and TNF-α in lipopolysaccharide-activated human peripheral blood mononuclear cells. The ethanolic extracts of the bran of both black and sumac sorghum varieties significantly reduced 12-O-tetradecanoylphorbol acetate-induced ear oedema in mice at an activity level similar to that observed with indomethacin, a standard

anti-inflammatory drug. The anti-inflammatory effects observed with the sorghum brans correlated with their phenolic content and antioxidant activity.

In vivo studies in adult male Wistar rats fed a hyperlipidic diet enriched with tannin sorghum flour indicated the potential to reduce inflammation and oxidative stress (Moraes et al., 2012). This was demonstrated by an observed reduced expression of the inflammatory factor TNF-α in epididymal adipose tissue and lower levels of thiobarbituric acid reactive substances (TBARS) in the liver. Such effects were not observed with non-tannin sorghum genotypes used in the study and it was suggested that the high phenolic content and antioxidant activity of the tannin sorghum genotype may contribute to the observed effects.

Lee et al. (2013) reported that ethanolic extracts from tartary buckwheat seeds were able to prevent inflammatory injury in ethanol-induced and carbon tetrachloride-induced liver damage in mice and rats. The ethanolic extracts inhibited increases in levels of indicators of liver toxicity (serum aspartate transaminase, alanine transaminase and alkaline phosphatise) and reduced levels of inflammatory factors (TNF-α, IL-1β and IL-6). Administration of the ethanolic extracts also increased the activities of antioxidant enzyme (catalase, glutathione peroxidase, glutathione reductase and superoxide dismutase). The authors suggested that these observed anti-inflammatory effect of the extracts could be due to the major bioactive phenolics, namely rutin and quercetin, in the tartary buckwheat.

Cardiovascular disease prevention and anti-hypertensive properties

Oxidation of low density lipoprotein (LDL) is believed to be a precursor for the development of coronary heart disease (Regnström et al., 1993). The oxidized LDL is taken up by macrophages (foam cells) (Jacob and Burri, 1996) and smooth muscle cells leading to the

formation of fatty streaks (Baba et al., 2007) and this leads to development of atherosclerosis. The prevention of LDL oxidation may therefore be a potential way to preventcardiovascular disease and hypertension. There are various reports concerning the ability of extracts from grains to prevent LDL oxidation by exerting antioxidant effects, presumably due to their content of phytochemicals such as phenolic compounds.

Buckwheat seed and buckwheat sprout meals have been shown to produce significant cholesterol lowering effects in hamsters (Lin et al., 2008). Meals suppressed LDL-cholesterol and elevated HDL-cholesterol which is important for prevention of cardiovascular disease. The authors suggested that hypocholesterolomic effects may be due to synergistic effects of high crude fibre and high rutin and quercetin concentrations (especially in eight day sprouts), which potentially suppresses cholesterol synthesis de novo.

Jiang et al. (2007) determined LDL oxidation inhibitory effects of extracts from three buckwheat species. Extracts from *Fagopyrum homotropicum* (0.8 mg buckwheat/ml) and *Fagopyrum tataricum* (0.2 mg buckwheat/ml) produced inhibitory effect on copper-induced LDL oxidation with average area under curve (AUC) values of 75.6% and 16.7%, respectively, compared to the control. The three buckwheat species exhibited a dose response effect on the AUC and lag time of LDL oxidation. There was a high correlation between the lag time and rutin content ($R^2 = 0.976$) and the lag time and total flavonoid content ($R^2 = 0.773$). There was an inverse correlation between AUC and rutin content ($R^2 = 0.971$) and AUC and total flavonoid content ($R^2 = 0.796$) in all buckwheat types. This study provides a clear link between rutin content and flavonoid content of the buckwheat grains and their ability to prevent LDL oxidation.

The effect of quinoa supplementation in fructose-containing diet on some cardiovascular disease-related parameters in male Wistar rats was studied by Pasko et al. (2010b). Rats fed quinoa had reduced serum total cholesterol, LDL, triglycerides, glucose and plasma total protein. Fructose feeding decreased HDL but this decrease was inhibited on feeding with quinoa. The reduction in LDL and prevention of decrease in HDL on feeding with quinoa suggests potential ability of the pseudocereal to prevent cardiovascular disease.

Letelier et al. (2011) showed the presence of thiol compounds and phenolics in a hydroalcoholic extract from quinoa seed coats. The extract inhibited rat microsomal lipid peroxidation which may be significant for prevention of LDL oxidation and hence prevention of atherosclerosis and cardiovascular disease. Oxidative stress-mediated conditions such as cardiovascular disease (Fearon and Faux, 2009) are also accompanied by loss of endogenous antioxidant compounds such as glutathione or decrease in glutathione-to-glutathione disulphide ratio (Schafer and Buettner, 2001). The extract inhibited microsomal lipid peroxidation induced by Cu^{2+}/ascorbate in a dose-dependent manner and also prevented loss of microsomal thiol content.

Anti-hypertensive properties are also determined by inhibition of angiotensin-1 converting enzyme (ACE). Angiotensin I-converting enzyme (ACE; peptidyldipeptide hydrolase, EC 3.4.15.1) catalyses the conversion of angiotensin I to angiotensin II, which is a vasoconstrictor, and subsequently increases blood pressure (De Leo et al., 2009). In addition, ACE degrades the peptide bradykinin, which has vasodilatory properties. The inhibition of ACE activity is therefore desirable in order to exert anti-hypertensive properties. Such ACE inhibitory activity has been demonstrated in vitro by extracts from day 2 buckwheat sprouts and day 10 seedlings (Randhir et al., 2008) and also in vivo (Nakamura et al. 2013). A

specially formulated food consisting of lactic acid fermented buckwheat sprouts produced blood pressure lowering effects in hypertensive rats (Nakamura et al., 2013). There were significant decreases in systolic and diastolic blood pressure in a manner similar to the anti-hypertensive drug captopril. Oral administration of the fermented buckwheat sprouts decreased ACE activity in the lung, thoracic aorta, heart, kidney and liver of the hypertensive rats. The fermented buckwheat sprouts also produced significant vasorelaxant activity.

In studies of the ACE inhibitory activity of ethanolic extracts (an indication of their anti-hypertensive properties) from pseudocereals and foxtail millet, Japanese millet and other true cereals, it was found that quinoa and buckwheat had higher ACE inhibitory activity (23%) than amaranth (9%) (Asao and Watanabe, 2010). Further, ethanolic extracts from the pseudocereals had higher ACE inhibitory activity than the true cereals,.

3. Biofortification

Biofortification aims to increase the density and bioavailability of key limiting nutrients, particularly micronutrients, in staple food crops such as grains (Welch and Graham, 2005; Nestel et al., 2006). There is compelling evidence that the consumption of such macronutrient and micronutrient biofortified cereals can alleviate malnutrition in developing countries. A meta-analysis showed that Quality Protein Maize (improve high lysine maize) consumption in place of conventional maize has substantially increased the rate of growth in weight and height of infants and young children with mild to moderate undernutrition (Gunaratna et al., 2011). Similarly, there are excellent calculated data that shows rice

biofortified with provitamin A (Golden Rice) could more than half the disease burden of vitamin A deficiency in India (Stein et al., 2008).

Three biofortification strategies have been identified: agronomic (increasing micronutrients through soil amendments or foliar application), conventional breeding (which includes induced mutagenesis) and recombinant DNA technology (genetic engineering, GM) (Carvalho and Vasconcelos, 2013). This review will only examine the latter two strategies. Since they involve genetic alteration to the grains, these technologies only require an upfront investment, and hence from a practical standpoint their implementation reduces recurrent costs to farmers (Poletti et al., 2004). The effects of biofortication on the end-use functional properties of these minor grains will be examined as well as the effects on their nutritional quality.

4.1 Macronutrients

Starch

In sorghum, the naturally occurring recessive waxy mutant (essentially 100% amylopectin, 0% amylose) has been known as early as the 1940s (Rooney and Miller 1982). Sorghum endosperm expresses up to three recessive waxy genes (*wxwxwx*), with heterowaxy mutants expressing one or two recessive genes (*WxWxwx* or *Wxwxwx*) and having intermediate levels of amylose (Sang et al., 2008). The high amylopectin trait is dependent on the absence of activity of the enzyme responsible for amylose synthesis, granule-bound starch synthase (GBSS) (Sattler et al, 2009). In sorghum, there are two classes of waxy mutants, classified according to the actual absence (*waxya*) or presence (*waxyb*) of the GBSS protein (Sattler et al., 2009). DNA lesions were found to be associated with the former and missense mutation,

leading to an amino acid substitution, with the latter (Sattler et al., 2009). Interestingly, it has been found that overall the waxy trait does not result in a yield penalty in sorghum hybrids (Rooney et al., 2005).

In vitro assay, and monogastric and ruminant animal feeding trials have consistently shown that waxy sorghum is more digestible and has higher feeding value than normal sorghum (reviewed by Rooney and Pfugfelder, 1986). In vitro studies also showed that waxy sorghum has a considerably higher proportion of rapidly digestible starch and a lower proportion of resistant starch compared to normal sorghum (Sang et al., 2008). Brewing trials with waxy sorghum as adjunct showed that the starch was hydrolysed more rapidly than normal sorghum, the wort had lower viscosity but was rich richer in complex carbohydrates (Figuero et al., 1995). Other work indicated that waxy sorghum adjunct gave higher extract yield, filtered more rapidly than normal sorghum and produced a wort with the same level of fermentable carbohydrates (Osoria-Morales et al., 2000). Similarly, a study of the effect of sorghum endosperm type on bioethanol production showed that waxy and heteroxy waxy lines had the highest efficiency (Wu et al., 2010).

There is also variation in starch digestibility in normal sorghum starch types. A recent report indicates that a low-frequency allele type (*SbPUL-RA*) in the starch metabolic gene, "pullulanase", is associated with increased starch digestibility in sorghum lines, regardless of genetic background (Gilding et al., 2013).

Concerning the millets, waxy, high amylopectin types also exist, including proso millet varieties from China (Graybosch and Baltensperger, 2009), East Asia (Hunt et al., 2012) and Japan (Araki et al., 2012) and in foxtail millet (Fukunaga et al., 2002). As with sorghum,

waxy proso millet shows higher fermentation efficiency in bioethanol production than normal proso millet (Rose and Santra, 2013). Waxy amaranth has also been identified and the trait has been found in three species of amaranth, namely *Amaranthus caudatus, A. cruentis* and *A. hypochondriacus* (Park et al., 2010) and is geographically widely distributed (Park et al., 2012).

Protein

In sorghum, like most cereals, lysine is the first limiting amino acid, but the lysine content of its protein is even lower, only 35-90% of that in the other cereals (Henley et al., 2010). By chemical mutagenesis using diethyl sulphate, a high-lysine sorghum mutant (P721-opaque) was developed, which has up to 60% higher lysine content, as a result of reduction in the relative amount of kafirin prolamin storage proteins (Guiragossian et al., 1978). However, an intervention trial with infants recovering from Protein-Energy Malnutrition revealed that diets based on both high lysine and normal sorghum gave similarly very poor nitrogen absorption and retention (MacLean et al., 1981), even compared with data from other cereals. In vitro work carried in parallel with this trial indicated that the poor quality of sorghum protein was not just due to its low lysine content but also to the low digestibility of the sorghum protein in foods (Axtell et al., 1981; Mertz et al., 1984).

To address this problem, scientists at Purdue University have developed novel sorghum lines with improved in vitro protein digestibility (25% higher in cooked flour) and somewhat higher lysine content by crossing the P721-opaque mutant with normal lines (Weaver et al., 1998) (Table 4). The higher protein digestibility of these lines was attributed to the fact that their protein bodies (the organelles of kafirin storage) are invaginated in shape, instead of being spherical (Oria et al., 2000). Recent research indicates that the invaginated shape of the

Table 4
Major ongoing sorghum and millet biofortification projects

Grain species	Project name Institution	Biofortified traits	Target levels	Levels achieved	Progress to 2013	Target region
Sorghum	Africa Biofortified Sorghum project Africa Harvest Biotechnology Foundation International	Essential amino acids	Lysine: 80-100% increase Tryptophan and threonine: 20% increase	Lysine: 75-97% Tryptophan: no data Threonine: no enhancement	All enhanced traits demonstrated but not all together in the same line Controlled field trials commenced	Sub-Saharan Africa
		Protein digestibility	60-80% increase	Raw: 61-83% Cooked: 43-59%		
		Iron and zinc bioavailability (phytate reduction)	30-50% increase	Iron: 6-20% (in vivo assay) Zinc: 25-39% (in vivo assay		
		Provitamin A	20-21 mg/kg	Up to 14 mg/kg		
Sorghum	Purdue University	High lysine and high protein digestibility	Not specified	Lysine: 60% Raw digestibility: 10-15% Cooked	Enhanced traits demonstrated Breeding ongoing	Africa and Asia?

				digestibility: 25%		
Sorghum	Texas A&M University	Combined waxy and high protein digestibility	Not specified	No data	Both traits demonstrated in the same line Varietal development commenced	USA
Sorghum	ICRISAT	Iron and zinc	Not specified	No data	Breeding commenced	India
Pearl millet	ICRISAT	Iron and zinc	Not specified	Iron: 67-73 mg/kg Zinc: 41-56 mg/kg	Varieties released	India and Africa

protein bodies are as a result of a single point mutation, rendering the single peptide of the 22kDa α-kafirin type resistant to processing (release from the protein body rough endoplasmic reticulum membrane) (Wu et al., 2013). The invaginations are proposed to facilitate access of digestive protease enzymes to the digestible α-kafirin protein located within the protein bodies, which is normally screened by digestion-resistant cross-linked kafirin involving disulphide bonding through the γ- and β-kafirin types (reviewed by Duodu et al., 2003). The sorghum high protein digestibility trait is thought to be due to a single recessive mutation (Winn et al., 2009). Interestingly, two close QTLs associated with protein digestibility have been found on the sorghum chromosome 1, one unfavourably affects protein digestibility and the other favours it (Winn et al., 2009). The authors proposed that lines with high digestibility result when the linkage between the two loci is broken.

Despite the improvement in in vitro protein digestibility of these high protein digestibility sorghum lines, animal feeding trails with pigs and broiler chickens did not show any improvement compared to maize or normal sorghum in important digestibility parameters (Nyannor et al., 2007). High protein digestibility sorghum flour has, however, been shown give better dough properties (resistance to extension and time to dough breakage) and higher bread loaf volumes than normal sorghum when composited with wheat flour (Goodall et al., 2012). Further, high protein digestibility sorghum flour was found to form a visco-elastic dough when mixed with vital gluten in an 82:18 ratio, whereas under the same conditions normal sorghum flour did not. They also have some better brewing attributes (Mugode et al., 2011). When malted, high protein digestibility had substantially higher levels of free amino nitrogen (FAN) than normal sorghums. However, their FAN production during mashing was not significantly higher.

The Africa Biofortified Sorghum (ABS) project, led by the Africa Harvest Biotechnology Foundation International (Biosorghum, 2010), has employed GM technology to improve sorghum, both protein lysine content and wet-cooked digestibility (Henley et al., 2010). RNA interference (RNAi) technology (Jung, 2008) is being used to suppress the synthesis of specific combinations of types of kafirin protein, as was first demonstrated with the maize zein prolamins (Segal et al., 2003). Additionally, where appropriate, transgenes for lysine-rich proteins such as HT12, an analogue of barley hordothionin, can be expressed (Zhao et al., 2003) and the catabolism of lysine by the enzyme lysine ketoreductase (LKR) suppressed (Grootboom, 2010). Early, transgenic events showed 45% and 76% increases in grain and endosperm lysine content, respectively (Grootboom, 2010). Improved lines with co-suppression of α-, γ- and δ-kafirins had double the content of lysine, 3.7-4.1 g/100 g protein compared to their parent lines 2.1-2.4 g/100 g, and a wet cooked in vitro protein digestibility of 81%, compared to its null control with 58% (Da Silva et al., 2011). The protein bodies of these improved protein quality sorghum lines were irregular in shape and surrounded by a dense matrix of protein and the endosperm was floury (soft). Lines where the synthesis of only the γ- and δ-kafirins was suppressed had normal shaped proteins bodies and corneous (hard) endosperm structure, but the improvement in protein digestibility was less. Thus, further improvements to obtain both improved protein quality and grain hardness are required. Notwithstanding this, a wide range of traditional staple African food products (thick porridge, alkaline porridge, flatbread, fermented flatbread and couscous) and western-style cookies of reasonable quality were produced from these GM improved protein quality lines (Taylor and Taylor, 2011). Significantly, all the products maintained their higher lysine content and protein digestibility compared to products made from their null controls and were higher in protein digestibility than the products made from a non-GM high protein digestibility line. The digestibility of the GM high protein sorghum was also found to have

useful characteristics for brewing and bioethanol production (Kruger et al., 2012b). When lines were used as whole grain adjunct, they yielded substantially higher extract and higher FAN than their normal sorghum null controls.

An alternative GM strategy for improving sorghum protein digestibility is to engineer it to express the NADP thioredoxin system with the aim of preventing/breaking the disulphide bonded kafirin cross-linking (Sahoo et al., 2007). Using this approach, a line which expressed the enzyme NADP-thioredoxin reductase showed an 11% increase in protein digestibility compared to its non-transgenic control (Hoang, 2008).

To improve the overall digestibility of sorghum, researchers are now studying lines that combine both the non-GM high protein digestibility and waxy traits (Wong et al., 2009) (Table 4). Interestingly, in a comparison of normal, high digestibility, waxy and waxy-high digestibility lines, some of the latter were among the best yielding, suggesting that it is possible to breed agronomically acceptable cultivars (Jambala et al., 2012). They also show promise for bioethanol production, having easily pasted starch granules, yielding higher free amino nitrogen, giving faster fermentation and producing lysine-rich distillers dry gain and solubles (DDGS), compared to normal sorghum lines (Wu et al., 2010).

4.2 Micronutrients

Provitamin A

Some sorghum varieties indigenous to West Africa (Nigeria and Niger) have a yellow endosperm, which is pigmented by carotenoids (Salas Fernandez et al., 2009). The most abundant carotenoids were found to be lutein, zeaxanthin and β-carotene. The highest level

of carotenoids found in a survey of 164 landraces was up to 1.7 mg/kg, which is, however, some two to six times lower than in yellow maize. Another study of eight yellow endosperm sorghum types, however, found lower levels of total carotenoids, only 0.1-0.3 mg/kg, with zeaxanthin being the most abundant and only modest amounts of β-carotene, approx. 6-150 µg/kg (Kean et al., 2007). With β-carotene-biofortified Golden Rice, the conversion factor for β-carotene to retinol has been calculated to be on average 3.8 to 1 by weight (Tang et al., 2009). Thus, at the levels found in the above studies, yellow endosperm sorghum would not a make significant contribution to meeting the human vitamin A requirements, where, for example, the recommended daily retinol safe limit intake is 450 µg/kg body weight for a 4-5 year old child (FAO/WHO, 2002).

However, another survey of 11 sorghum yellow endosperm lines showed much higher levels of β-carotene, 0.6-1.1 mg/kg (Reddy et al., 2005). Comparisons of sorghum carotenoid content data are very problematical because of differences in analytical methodology (Salas Fernandez et al., 2009) and the fact that carotenoid levels decline significantly during grain maturation (Kean et al., 2007). Further, the carotenoid pigments oxidise when exposed to light, with large differences being found between decorticated sorghum flour that was stored bagged versus unbagged, 9.9-13.7 mg/kg and 2.9-7.2 mg/kg, respectively (Kean et al., 2011). Notwithstanding these issues, when porridges were made from these yellow endosperm sorghums and yellow maize it was found that the carotenoid bioaccessibility (an in vitro measure of bioavailability) was generally higher in the yellow endosperm sorghums and that the xanthophylls (lutein and zeaxanthin) showed higher micellarization (the measure of bioaccessibility) was higher than for the β-carotene (Kean et al., 2011). This indicates that the xanthophylls in yellow endosperm sorghum are also a source of vitamin A.

QTLs for the yellow endosperm trait and carotenoids in sorghum have been identified (Salas Fernandez et al., 2008). Colour QTL co-localised with carotenoid QTL. Five QTLs for β-carotene were identified, with one on chromosome 2 being stable across environments and explaining a large proportion of phenotypic variance. Perhaps most importantly, it was associated with a new phytoene synthase gene (*Psy3*).

The ABS project is using GM technology to develop a transgenic biofortified provitamin A "golden sorghum" (Pioneer Hi-Bred, n.d.; Lipkie et al., 2013) with β-carotene levels of up to 21 mg/kg (Saltzman et al., 2013) (Table 4). The aim is to meet 30-50% of a child's recommended dietary allowance (Wambugu et al., 2012). The technology being used to develop the "golden sorghum" is similar to that of Golden Rice, where transgenes coding for enzymes involved in β-carotene synthesis, phytoene synthase (*psy*) and carotene desaturase (*crtI*) are key components (Paine et al., 2005). However, specific sorghum promoters are being used (Lipkie et al., 2013). The GM sorghum lines were found to contain substantially higher β-carotene (3.3-14.0 mg equiv. /kg) compared to that in the germplasm background (1.0-1.5 mg/kg) (Lipkie et al., 2013). The micellarization efficiency of the β-carotene in porridges was, however, lower in the GM lines (1-5%) compared to the null/non-GM sorghum controls (11-16%). It was suggested that the transgenic modifications adversely affected carotenoid bioaccessibility through localisation and sequestration. Notwithstanding this, one line, Homo188-A, had a 4-8 fold higher bioaccessible β-carotene compared to the controls. This suggests that the application of GM technology to substantially increase the β-carotene content of sorghum can enhance the level of bioavailable provitamin A in foods made from sorghum.

A survey of 54 pearl millet types (landraces, varieties and lines) showed that the levels of β-carotene did not vary greatly and were quite low 48-69 µg/kg (Buerkert et al., 2001), but evidently of the same order of magnitude as reported for yellow endosperm sorghum (Kean et al., 2007).

Concerning the pseudocereals, cultivation trials of 27 quinoa lines showed that their total carotenoid content ranged from 1.7 to 5.5 mg/kg (Bhargava et al., 2007). Importantly, carotenoid content had 98% heritability component and a genetic gain of 65% against the mean value was obtained.

Minerals

Mineral biofortification has focussed on iron and zinc, as these are the most limiting mineral micronutrients in the plant-based diets of many people in developing countries (Hunt, 2003). Concerning sorghum, cultivation trials of 29 accessions in the ICRISAT (International Crops Research Institute for the Semi-Arid Tropics) core collection showed significant genotype differences for zinc content but not for iron (Ashok Kumar et al., 2009). However, iron and zinc contents were positively correlated ($p<0.01$). Five lines with both high iron (>50 mg/kg) and zinc (>37 mg/kg) contents were identified. Inheritance of iron and zinc content in sorghum has been been studied in more detail (Ashok Kumar et al., 2013). It was found that through exploiting heterosis, it is possible to improve iron content, but not zinc content. To develop hybrids with high iron and zinc content, both parents will need to be improved for these minerals. Notwithstanding this, it was concluded that it is feasible to combine higher iron and contents with high yield. ICRISAT is now developing zinc and iron biofortified sorghum (Saltzman et al., 2013) (Table 4).

ICRISAT is also leading a project to develop iron and zinc biofortified pearl millet through selection and conventional breeding (Rai et al., 2012; Saltzman et al., 2013) (Table 4). Cultivation trials of 120 lines revealed considerable variability in iron (30 to 76 mg/kg) and zinc (25-65 mg/kg) contents and that their contents were significantly ($p<0.01$) correlated (Velu et al., 2007). Highest levels were found in well-adapted commercial varieties and also in a particular germplasm (*Iniari*). The significant correlation between pearl millet iron and zinc contents has subsequently been confirmed in several cultivation trials (Rai et al., 2012). However, it was also found that there were significant negative correlations ($r = -0.39$ to $r = -0.58$) between iron content and grain yield in three of six trials. Another potential problem is that, with a few exceptions, hybrids did not outperform parents with high iron and zinc contents, indicating that there is little or no opportunity to exploit heterosis (Velu et al., 2011).

Since foods from cereals such as pearl millet and sorghum are generally made from decorticated (debranned) grain (Murty and Kumar, 1995), it is important to know what the effects of decortication are on the levels of iron and zinc in such biofortified cereals. Two iron and zinc biofortified pearl millet varieties and a traditional Burkina Faso variety were subjected to decortication and mineral bioavailability was estimated in terms of the molar phytate: mineral ratio (Hama et al., 2012), as phytate inhibits pearl millet mineral availability (Lestienne et al., 2005). It was found that although there was a decrease in mineral content with extraction rate in all three varieties, the biofortified varieties maintained their substantially higher iron and zinc contents down to at least a 70% extraction rate (probably the maximum used in practice) (Hama et al., 2012). With zinc, the phytate: zinc ratio was much lower for the biofortified varieties (<18:1) than the traditional variety at all extraction levels, a ratio that corresponds to moderate bioavailability (Gibson, 2006). The phytate: ratio

for iron of the biofortified varieties, although lower, was, however, considerably >1, indicating no improvement in iron bioavailability. Notwithstanding this, recent research showed that when the iron and zinc biofortified pearl millet was fed to 2-year old children in the form of porridges and flatbread , the quantity of these minerals absorbed was more than adequate to meet the children's requirements (Kodkany et al., 2013). Unfortunately, the study did not reveal whether the pearl millet had been decorticated.

4.3 Antinutrients

Phytate

An alternative strategy is to improve the availability of the minerals present in the grain through genetically reducing the level of phytate (myo-inositol hexakis phosphate) (reviewed by Brinch-Pederson et al., 2007; reviewed by Raboy, 2009; reviewed by Rawat et al., 2013). Phytate chelation of mineral cations can severely adversely the bioavailability of essential minerals, particularly zinc, iron, calcium, magnesium and to a lesser extent manganese (reviewed by Lönnerdal, 2002; reviewed by Kumar et al., 2010). The ABS project aims to increase iron and zinc in sorghum by 30-50% by reducing its phytate content using GM technology (Grand Challenges in Global Health, 2013) (Table 4). Two approaches are being investigated: 1) modulation of the expression of myo-inositol kinase (MIK) (Shi et al., 2008; Kruger et al., 2012a), which is responsible for myo-inositol phosphorylation (Shi et al., 2005); and 2) modulation of phytate synthesis utilising multidrug resistance-associated protein (MRP) nucleotides to modulate expression of MRPs (Shi et al., 2011; Kruger et al., 2013). Mutations in the MRP transporter gene 5 (a member of the ATP-Binding Cassette (ABC) transporter gene family) which silence expression of this transporter, have been found to dramatically reduce phytate in cereal grains and legume grains (Shi et al, 2007; Xu et al., 2009; reviewed by Raboy 2009). Importantly, it has been found that in maize, the resulting

low phytic acid-high inorganic phosphate seeds germinated normally and did not have a significant reduction in seed weight (Shi et al., 2007).

Low-phytate ABS sorghum (non-tannin and tannin lines) with phytate reduced by the "MIK" technology showed a 32-46% phytate reduction compared to their null controls in whole grain and unfermented and lactic acid bacteria fermented porridge products (Kruger et al., 2012a). The modification in combination with fermentation resulted in a substantial improvement in in vitro iron availability (dialysability bioaccessibility assay) in porridges made from the non-tannin lines (30%), compared to 10% for unfermented porridges made from the low-phytate lines and 18% in fermented porridges made from the null controls. With the low-phytate, tannin sorghum lines, there was no improvement in iron bioaccessibility, presumably because tannin binding of the iron (Towo et al., 2006) overwhelmed any phytate reduction effect. The non-tannin low-phytate sorghum lines also showed improved mineral solubilisation during the brewing/bioethanol production mashing process (Kruger et al. 2012b), suggesting their potential to improve yeast nutrition during fermentation.

Low-phytate ABS sorghum (non-tannin lines) with phytate reduced through genetic modifications of the MRP transporter genes showed a substantially greater reduction in phytate content in the whole grain (80-86%) (Kruger et al., 2013). This resulted in improved iron availability in vitro (dialysability and Caco-2 cell uptake) and in vivo (suckling rat pup absorption), of between 37-61%, 3-16% and 6-20%, respectively in whole grain porridges made from the low-phytate sorghum lines, compared to their null controls. Zinc in vivo absorption and uptake by Caco-2 cells were also improved, by 13-29% and 25-39%, respectively.

Tannins

The negative effect of tannins in sorghum on mineral availability has been indicated above. Recently, the genetics governing the presence or absence of tannins in sorghum have been elucidated (Wu et al., 2012). Nucleotide polymorphisms in the *Tannin1* gene, coding for a WD40 protein are involved. Deletions or insertions in the coding region result in non-functional alleles, *tan1-a* and *tan1-b*, respectively. This discovery provides the basis for better control of tannin expression to minimise tannin antinutritional effects but maintain the agronomic benefits of tannins in sorghum. Some varieties of finger millet also contain condensed tannins (Siwela et al., 2007). Presumably, the genetics of their presence or absence is similar.

3. Protein functionality for quality baked goods

Before considering the role of proteins in these products it is important to ask what the market is for such products and what the consumer actually wants from them. There are two basic classes of product: 1. gluten-free products, 2. products containing both gluten and another flour from a cereal or pseudocereal that does not contain gluten. "Gluten" in this context is any form of prolamin that may induce an undesirable reaction in humans and includes wheat, barley or rye and possibly oat prolamins. In the case of gluten-free products the driving need is medical. Patients with coeliac disease or other forms of unfavourable reaction to gluten have no choice but to avoid it (Arendt and Dal Bello, 2008; Rossell, 2009). However, they wish to enjoy the convenience of such products and their ready availability in catering outlets as a well as in the home.

In the case where there is only partial replacement of gluten the possible reasons for production and consumption are more varied. There may a health benefit to be gained in terms of amino acid or other chemical content or there may be a market for baked products with novel flavours and textures. Alternatively, particularly in developing countries, there may be a wish to incorporate local protein sources rather than expensive imported wheat. The health benefits themselves come in two categories: for populations with limited food availability governments may wish to add proteins which have nutritional benefits, or, in richer countries consumers may wish to buy products which can make health claims.

The reasons for producing baked products with no or reduced gluten may therefore be divided into two categories: a needs driven category, where there is some medical or economic imperative, and market opportunity category. In the latter case the consumer may well not want a product that resembles the traditional white loaf and may want novel flavours and textures. An example of this is the rise, in economically developed countries, of the sales of "artisan" breads which come in variety of colours, textures and tastes. It is also probable that consumers of these types of products would also wish them to be made using a traditional process. In the needs driven category the problem is one of substitution, not new product opportunities. The end product must therefore be very similar to the traditional baked product. Thus, the process is constrained firstly by the need to produce a dough from the wheat component and secondly by the cost of manufacture which must be less than or equal to the target traditional product.

In general it is not obvious that the route to the final product must be similar to the traditional route. The problem is not one of producing a baked product by a process akin to the

traditional baking process but reverse engineering a baked product. That is producing a food that has the appearance taste and texture of the target. As an example, consider a possible approach to making gluten-free bread. A three dimensional image could be constructed of the loaf. Using a 3-D printer it would be fairly straightforward to reproduce the structure in a suitable plastic. However, current developments in 3-D printing with biopolymers (Lam et al., 2002; NASA, 2013) suggest that it might be possible to carry out such a process with a mix of protein and starch. The structure would then be built in in the printing process and the problem then becomes one of ensuring that the mechanical properties of the matrix are suitable and flavour an appearance are appropriate. This process may not be practical either technologically or economically, but the point is that the required outcome is the product and not the process in this case.

Before considering any process it is important to pay attention to the nature of the proteins to be used. These fall into two classes: the prolamins, which are present in cereals and a predominance of globulins with some albumins in pseudocereals (Shewry, 2002). The proteins belong to different major groups and have significantly different structures and properties. Globulins may be classified into groups depending on their sedimentation coefficients (which reflect their molecular masses). Typical 11S globulins are hexameric with a molecular weight of the order of 250-400k. The subunits consist of two chains which are basic and acidic and liked by single disulphide bond. The actual details vary from plant to plant (Shewry, 2002) and there may be formation of decamers or monomers depending on pH, temperature or ionic strength. They may also form gels, such as tofu from soybean, on heating (Mills et al., 2003). 7S globulins are trimeric and have molecular weights in the order of 150-190k and have no disulphide bonds. Albumins typically have a molecular weight of 8-15k and contain a small and a large subunit, which are linked by two disulphide bonds. More

detail of molecular weight and structure is given in Table 5. The prolamins, which only occur in grasses, are much more diverse. They are rich in glutamine and proline and are soluble in alcohol water mixtures. Table 5 gives a comparative list of the prolamins present in wheat gluten and kafirin from sorghum with an indication of the main structural elements. The structures of the millet prolamins seem to be very similar to those of kafirin (Adebowale et al., 2011; Belton et al., 2006; Bugs et al., 2004).

In wheat doughs the main determinants of the rheological properties are the group of prolamins known as high molecular weight glutenin subunits. These respond to mechanical extension by transforming their β–turn structure to β–sheet structures. Mechanical work in the presence of air also allows the reformation of disulphide linkages between proteins resulting in more favourable interchain interactions. This allows increased resistance to deformation on repeated extension and finally the correct rheological properties to contain expanding gas bubbles (Belton, 2005). Given the structural differences between wheat prolamins and the other prolamins and globulins would seem that it is very unlikely that they could participate in suitable dough formation in a positive way. This is indeed the case (Hager et al., 2012b; Houben et al., 2012; Huttner and Arendt, 2010; Zannini et al., 2012) and the main technological problem faced in wheat gluten replacement is that any addition of a flour from a non-wheat source results in a reduction in product quality. This is not merely due to differences in protein structure but also to significant differences in the behaviour of starch. Even if protein behaviour could mimic that of gluten it is by no means certain that flour containing this and non-wheat starch would result in suitable baked products.

Much of the research on the use of alternative cereals and pseudocereals has used a variant of the dough method of production in which all the components are placed together with water

Table 5

The main seed storage proteins of proteins of wheat, sorghum and pseudocereals. Data compiled from Belton et al. (2006), Marcone et al. (2006) and Shewry (2002)

Plant Type	Protein	Fraction of total (%)	Main structural elements	Molecular weight of monomer/10^3	Main amino acids	Cysteine content (Mol%)	Polymer/monomer
Wheat	High molecular weight subunits	6-12	β turn, β sheet	65- 90	GLN, PRO GLY	0.5-1.5	polymer
	Low molecular weight subunits, Alpha and gamma gliadins	70-80	α helix, β sheet	30-40	GLN, PRO	2-3	polymer (LMW) Monomer (gliadins)
	omega gliadin	10-20	β sheet, β turn	40-75	GLN, PRO, PHE	0	Monomer
Sorghum	alpha kafirin	80	α helix	26-27	GLN, PRO, ALA, LEU	1	Monomer
	beta kafirin	7-8		16-20	GLN, PRO, ALA, LEU	5	Polymer
	gamma kafirin	9-12		20	GLN, PRO, ALA, GLY, LEU	7	Polymer

Plant Type	Protein	Fraction of total (%)	Main structural elements	Molecular weight of monomer/10^3	Mmain amino acids	Cysteine content (Mol%)	Polymer/monomer

mixed, proved and then cooked (Arendt and Dal Bello, 2008; Arendt et al., 2008; Berghofer and Schoenlechner, 2009; Hager et al., 2012b; Houben et al., 2012; Schoenlechner et al., 2013; Taylor and Emmambux, 2008). The production of gluten-free bread has been reviewed (Alvarez-Jubete et al., 2010a; Taylor and Emmambux, 2008; Taylor et al., 2006; Zannini et al., 2012). In situations where the main component of the product is wheat flour there is little alternative to using a mixing and proving method similar to that used for 100% wheat products. However, there is some evidence that pre-treatment of sorghum by fermentation or malting can improve the bread making properties (Hugo et al., 2003; Hugo et al., 2000). In the case of gluten-free recipes, the water content is such that a batter rather than a dough is formed but the process typically followed is one of adding and weighing all ingredients, mixing, proving and baking. This procedure demands that the mixture formed must have good bubble holding capacities and maintain the appropriate rheology throughout the process. As pointed out above, given the huge differences between the protein structure of wheat gluten and any other protein simply using substitutes from whatever source is not likely to give the desired result. The approach has been mainly been to attempt to improve the behaviour of the batter/dough by the use of a variety of processing aids and pre-treatments (Angioloni and Collar, 2012; Hager and Arendt, 2013; Hager et al., 2012a; Renzetti and Arendt, 2009; Rossell, 2009). Most of this work has not been informed by a consideration of the molecular properties of the proteins or other biopolymers involved, but has used an empirical approach based on measurements of batter rheology or systematic variation of ingredient ratios.

A notable exception to this approach has been the work of Hamaker's group (Erickson et al., 2012). They noted that in sorghum the protein bodies are difficult to disrupt and therefore in normal sorghum flour the protein is effectively inert as it is contained in the protein body

(Goodall et al., 2012). However, in the case of the high protein digestibility mutant, the protein bodies have substantially lost their integrity (Oria et al., 2000) and the protein is much more available. Using this mutant it has been shown that doughs enriched with wheat gluten showed better rheological properties than similarly enriched doughs from normal sorghum and the resulting bread had higher loaf volume (Goodall et al., 2012).

Other work showed that application of shear to zein above its glass transition temperature increased the amounts of β–sheet structure formed in the protein (Mejia et al., 2007). On removal of shear the structure rapidly disappeared. In the β–sheet form the viscoelasticity of the polymer was high and decreased with decreasing β–sheet content. It was hypothesised that the effects seen were due to interactions similar to those responsible for the viscoelastic properties of the high molecular weight subunits of gluten (Belton, 2005). These effects may explain the observations of Schober and co-workers that ensuring that zein in the dough mixing process is above the glass transition temperature creates a system with a high viscoelasticity (Schober et al., 2008; Schober et al., 2011; Schober et al., 2010). Similarly it has been shown that mixing either zein or kafirin with plasticiser to form a dough-like resin results in a viscoelastic system (Oom et al., 2008).

Further work showed that addition of β–sheet rich protein to the mixed zein stabilised the β–sheet content and stabilised increased viscoelasticity (Mejia et al., 2012). This effect was observed with additions of casein as low as 3% and has been termed “co-protein effect”.

It might be argued that the globulins from pseudocereals already have considerable β–sheet content and therefore should be able to form good doughs. However, the β–sheet in this case is intramolecular and is mainly associated with a β–barrel structure (Tandang-Silvas et al.,

2012) that appears to be very stable with denaturation temperatures for amaranth and buckwheat globulins around 95°C (Marcone et al., 1998). Thus, the β–sheet structure is intra- rather than intermolecular and cannot contribute to protein-protein interactions.

The work of Hamaker's group has significant implications. It suggests that under the right circumstances it might be possible to "unravel" a protein so that it has little structure and the possibility of protein/protein interactions by intermolecular β-sheet formation is enhanced. The stabilisation of the β-sheet form by addition of other β-sheet proteins (Fevzioglu et al., 2012) is very promising in consideration of baked products containing a mixture of wheat flour and a minor grain protein source. The high concentration of β–sheet already in the wheat gluten should readily stabilise a suitably prepared protein form.

4. Conclusions

Research has shown that sorghum, millets and pseudocereals are generally very rich in phenolic phytochemicals, which may have important health-promoting properties. Because of this, these minor grains should be a component of everyone's' diet, thereby increasing people's dietary diversity and maintaining food plant biodiversity. Future research into elucidating the effects of consuming these grains on long-term health needs to be more food-related. People do not eat organic solvent extracts. Macro- and micronutrient biofortification of sorghum and pearl millet is making substantial progress and some of the lines have good agronomic characteristics and useful commercial end-use attributes. Both of these properties are essential, otherwise in developing countries people will increasingly cultivate the major grains in preference. To achieve the conflicting requirements of nutritional and end-use

quality and agronomic improvement, the power of recombinant DNA and related genomic technologies to dramatically reduce breeding time should be harnessed. Concerning their use in staple dough-based foods such as bread for the burgeoning urban population in developing countries and for "gluten-free" applications, it appears that understanding the molecular structure of the storage proteins of the minor grains and manipulating their β-sheet structure are key to achieving what has been described as the Holy Grail of gluten-free breads, "forming a wheat-like dough from non-wheat proteins" (Durham, 2010).

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