Millet grain as a candidate antioxidant food resource: a review
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ABSTRACT
Millet is a major drought-resistant crop that serves as a nutrient rich food staple in Africa and Asia. In addition, millet contains an abundance of bioactive compounds with antioxidant activity. The intake of antioxidants through the diet is essential for improving human health. This review aimed to evaluate the antioxidant compounds in millet, as well as the factors that influence antioxidant activity. The millet contained several natural occurring phenolic compounds which include phenolic acids, flavonoids and tannins, in addition to xylo-oligosaccharides (XOs), insoluble fibers and peptides. Certain lipophilic antioxidants, including vitamin E and carotenoids, were extensively distributed among varieties. Furthermore, the bioactivity of the antioxidants can be affected by food processing. Germination and fermentation could enhance the antioxidant properties, due to increased antioxidants content (phenolic compounds). In summary, it is possible to use millet as a nutraceutical and antioxidant food resource to reduce disease risks and maintain health.

ARTICLE HISTORY
Received 2 April 2019
Revised 28 August 2019
Accepted 6 September 2019

KEYWORDS
Millet; Antioxidant activity; Phenolics; Processing

Introduction
Drought-tolerant grains are excellent choices for several populous countries with water shortages such as those in central Africa and Asia. Millet is a superior drought-resistant grain and the sixth most economically significant global agricultural crop. In addition, millet is highly resistant to pests and diseases, exhibits a rapid growth rate, can flourish in less fertile soil, and generates a high yield even in adverse under heat and drought settings compared to other major cereals. Millet belongs to the family Poaceae (previously called Gramineae) and consists of several varieties. Pearl millet (Pennisetum glaucum) is a significant variety, representing 40% of the global production, followed by other varieties such as foxtail millet (Setaria italica), proso (or white) millet (Panicum miliaceum), and finger millet (Eleusine coracana). Other millet varieties include kodo millet (Paspalum setaceum), little millet (Panicum sumatrense), and barnyard millet (Echinochlos utilis). The total global millet production in 2016 was estimated at approximately 28 million tons, with Africa and Asia serving as the producers, respectively generating 48% and 47% of the total global yield.

Except for lysine and threonine, millets proteins are excellent sources of essential amino acids, while the grains are also rich sources of phytochemicals and micronutrients. Aside from its nutritive value, millet is also recognized for its numerous potential health benefits, which include the enhancement of wound healing, the prevention of cardiovascular disease, and decreasing blood glucose and cholesterol levels. A previous study postulated that oxidative stress might cause various chronic conditions, including cardiovascular disease, cancer, neurodegenerative disorders, arthritis and diabetes. Antioxidants are thought to be important in reducing oxidative damage. Previous phytochemical profiling of millet...
indicated that it contained significant amounts of antioxidants such as carotenoids, phenolics, and tocopherols.\textsuperscript{[13]} These findings have increased interest in using millet grains as a food, source due to its potential for being used to produce healthy value-added products. This review described the various antioxidant compounds that had been reported in millets as well as the factors that influence antioxidant activity.

**Chemical and nutritional composition of millet**

Millet consists of layers of husk and bran, which the husk contributes to 13.5\% of the foxtail millet content, whereas the bran and the germ comprises 1.5-2\%.\textsuperscript{[5]} The edible portion of the millet consists of carbohydrates (60-70\%), proteins (7-11\%), and fat (1.5-5\%), as well as minerals and vitamins.\textsuperscript{[14]} Millet proteins, are rich in essential amino acids. Mohamed et al.\textsuperscript{[15]} reported that foxtail millet was richer in lysine than most cereals and could be used as a supplemental protein source. The content of essential amino acids (leucine, isoleucine, and methionine) in proso millet was shown to be significantly richer than that of wheat.\textsuperscript{[16]}

Studies have indicated that millet exhibited an average fat content of 3.5–5.2\%, which was similar to that in maize yet higher than that reported in wheat and rice.\textsuperscript{[17]} Furthermore, research has demonstrated that the fat digestibility of millet was considerably higher compared to corn,\textsuperscript{[18]} while the fat also contained a higher level of unsaturated fatty acids.\textsuperscript{[19]} Linoleic acid was shown to be the predominant fatty acid in foxtail millet comprising 66.68\% of its total fatty acid content, followed by byoleic acid, palmitic acid, stearic acid, and linolenic acid (16.11\%, 7.42\%, 6.84\%, and 2.48\%, respectively).\textsuperscript{[20]} Millet was also found to contain high amounts of micronutrients such as minerals and vitamins. The levels of millet minerals such as magnesium, manganese, and phosphorus were significantly higher than in other cereals.\textsuperscript{[21]} Finger millet was shown to be rich in calcium with approximately 350 mg/100 g.\textsuperscript{[17]} Research indicated that the vitamin E and carotenoid content of foxtail millet could reach up to 31.36 mg/100 g and 0.19 mg/100 g, respectively.\textsuperscript{[22]}

Besides its nutritional value, components of millet possess various biological activities that are beneficial to health. A previous study indicated that proteins from proso millet could improve glycemic responses, as well as insulin levels in genetically obese mice with type-2 diabetes in high-fat feeding conditions.\textsuperscript{[23]} Aqueous extracts derived from foxtail millet grains have been reported to exhibit superior anti-hyperglycemic activity.\textsuperscript{[24]} Another study involving hyperlipidemic rats reported that foxtail and proso millet could decrease serum triacylglycerol levels, whereas foxtail millet reduced C-reactive proteins levels, which were related to inflammation responses, thereby suggesting that these grains might potentially be utilized in preventing cardiovascular disease.\textsuperscript{[10]}

**Antioxidant compounds in millet**

Numerous compounds in millet exhibited antioxidant properties (Table 1). The phytochemicals in millet, such as phenolics and dietary fiber, were predominantly posited in the bran layers, together with micronutrients (carotenoids and tocopherols) known to have antioxidant properties. Moreover, millet could also be enriched with antioxidants (such as peptides) via fermentation and germination.\textsuperscript{[37,38]}

**Phenolic compounds**

The primary phenolic compounds found in cereals included phenolic acids and tannins, whereas flavonoids occurred in small quantities,\textsuperscript{[39]} and have been shown to act as reducing agents or free radical terminators, metal chelating agents, and single-oxygen molecule quenchers.\textsuperscript{[26]} The phenolic content differed among millet varieties and grain fraction. Furthermore, phenolic antioxidant activity depended on phenolic ring position, and the degree of hydroxylation.\textsuperscript{[40]} Various other structural features further influenced the antioxidant activity in millet grains.\textsuperscript{[41]}
The millet predominantly contained phenolic acids in the bound forms (60%), whereas the rest occurred as free molecules.\textsuperscript{[42]} Hydroxycinnamic acids, including its derivatives, were the most common phenolics and were present in the insoluble-bound fractions of the phenolic acids from the millet grains.\textsuperscript{[43]} Ferulic acid, a type of hydroxycinnamic acid and a powerful antioxidant, exhibited high levels of free radical scavenging as well as anti-inflammatory activity.\textsuperscript{[44]} Besides the monomeric compounds, ferulate dimers exhibiting higher antioxidant activity, were present in the millet grains.\textsuperscript{[28]} Cereal ferulic acids displayed strong antioxidant activity when occurring in their bound form. Therefore, these did not require digestion with the assistance of microbial activity to facilitate their release within the colon\textsuperscript{[45,46]} Water-soluble feruloyl arabinoxylans, also known as feraxans, derived from finger millet displayed exceptionally strong antioxidant activity, approximately 5000-fold higher than that of sulfated polysaccharides.\textsuperscript{[46]} In addition to phenolic acids, sugars with > C = O (uronyl/acetyl) moieties and their level or nature of polymerization significantly enhanced the antioxidant activity during this process. Furthermore, the antioxidant ability of free phenolic acids was influenced by the number and location of hydroxyl groups on the aromatic ring, as well as the type of substituent, while it was positively correlated to the number of phenolic hydroxyl moieties.\textsuperscript{[47]} Moreover, the substitution of hydroxyl groups in the ortho position with electron-donating groups, which included methoxyl groups, also enhanced antioxidant activity.\textsuperscript{[48]} 

Flavonoids are the largest and most heterogenous group of plant phenolic compounds, and are abundant in fruits and vegetables, as well as in grains. Various flavonoids, which included anthocyanidins, chalcones, aminophenolics, flavanols, flavones, and flavanones were present in the millet.\textsuperscript{[28]} Flavonoids occurred in conjunction with sugars, known as glycosides of the O- or C- forms, although these might also be distributed as free aglycones.\textsuperscript{[49]} However, flavones-C-glycosides were the predominant flavonoids in the major cereals.\textsuperscript{[50]} The flavones, luteolin, and tricin were isolated from Japanese barnyard millet grains and were shown to have antioxidant activity.\textsuperscript{[51]} Whole millet grains as well as pearled fractions, displayed high levels of flavonoids, and have been utilized as functional food ingredients in basic diets.

| Name of compound | Major types | Mechanisms of Action | References |
|------------------|-------------|----------------------|------------|
| Phenolic acids   | Kodo, Finger, Foxtail, Proso, Little, Pearl millet | Their ability to donate hydrogen atoms via hydroxyl groups on benzene rings to electrophilic free radicals and in turn form a resonance-stabilized and less reactive phenoxyl radical. | [25–30] |
| Ferulic acid     | Kodo, Finger, Foxtail, Proso, Little, Pearl millet | Multiple hydroxyl groups confer upon the molecule substantial antioxidant activity. A double bond and carbonyl function in the heterocycle or polymerization of the nuclear structure increases its activity by affording a more stable flavonoid radical through conjugation and electron delocalization. | [31,32] |
| Tannins          | Finger millet | Procyanidin o-quinone is capable of producing oligomeric compounds through various coupling reactions that retain the number of hydroxyl groups, and that can act as prooxidants by forming reactive oxygen species through futile redox cycling | [32,33] |
| Xylo-oligosaccharides | Finger millet | Most oligosaccharides consist of ester linked phenolic acids. Apart from phenolic acids, the presence of sugars with > C = O (uronyl/acetyl) groups and degree/nature of polymerization impart strong antioxidant activity to the polysaccharides | [26] |
| Insoluble fibers | Foxtail millet | The antioxidant properties of insoluble fiber could be attributed in part to their unique phytochemical composition | [31,34] |
| Protein and Peptides | Peal, Foxtail millet | The antioxidant activity of proteins is due to complex interactions between their ability to inactivate reactive oxygen species, scavenge free radicals, chelate prooxidative transition metals, reduce hydroperoxides, enzymatically eliminate specific oxidants, and alter the physical properties of food systems in a way that separates reactive species | [1,15,35] |
| Carotenoids      | Finger, Little, Foxtail, Proso millets | Carotenoids act as antioxidants by quenching single oxygen and free radicals | [27] |
| Vitamin E        | Finger, Little, Foxtail, Proso millets | Biological activities of tocols are generally believed to be due to their antioxidant action by inhibiting lipid peroxidation in biological membranes | [13,36] |
Among the naturally occurring antioxidants, high-molecular weight tannins were reported to exhibit superior in vitro antioxidant activity. McDonough et al. found an appreciable level of tannin in some millet varieties, especially in some colored varieties. It has been suggested that condensed tannins were substantial contributors to the grain color in finger millet. Brown finger millet possessed higher antioxidant properties compared to white grains. Unlike grains that do not contain tannins, finger millet types that are rich in tannins, particularly those in the condensed form, exhibited higher antioxidant activity.

Xylooligosaccharides (XOs) and insoluble fibers

The XOs was composed of D-xylene units and linked by β-1,4 glycoside bonds, which have low degree of polymerization (DP 2–10) and might include xylobiose, xylotriose, and xylotetrose. The majority of oligosaccharides consist of ester linked phenolic acids such as ferulic, coumaric and caffeic acids. XOs are produced by hydrolysis reactions involving arabinoxylans derived from cereal and millet brans. The high antioxidant activity of ragi. XOs might be attributable to the significant increase in ferulic and syringic acid content. In addition to phenolic acids, the sugars with the > C = O (uronyl/acetyl) moiety and high levels or the nature of polymerization render these polysaccharides elevated antioxidant activity.

The insoluble fibers in foxtail millet also exhibited antioxidant activity, with the yellow kind displaying higher amounts of antioxidants compared to the white variety. The antioxidant features of the insoluble fibers might may be partly attributable to their phytochemical composition. Polysaccharides derived from foxtail millet in a basic pH solution exhibited a higher capacity for scavenging 1,1-diphenyl-2-picrylhydrazyl (DPPH) and hydroxyl radicals.

Proteins and peptides

Antioxidative peptides derived from pearl millet was isolated using trypsin. The antioxidant activity, Fe²⁺ chelating ability (51.20%), hydroxyl activity (60.95%) and reducing power (0.375 nm) of this protein hydrolyzate was assessed using DPPH (67.66%) and 2,2’-azinobis-3-ethylbenzothiazoline-6-sulfonate (ABTS) (78.81%) assays. In addition, the antioxidant properties of the hydrolyzates from foxtail millet was similar to α-tocopherol (86.27%) yet higher than ABTS and DPPH and showed metal-chelating activity. Functional foods and nutraceuticals might, therefore, be supplemented with peptides that could prevent damage caused by oxidative stress in various human disorders. The His, Leu, Gly, and Pro amino acids have been shown to play a crucial role in radical scavenging. Peptide fractions obtained via the PR-HPLC purification of fermented Tyr/Leu-rich foxtail millet were reported to exhibit significant radical scavenging activity.

Carotenoids and Vitamin E

Carotenoids and tocopherols pertain to apolar of well characterized apolar antioxidants. The lipidsoluble properties of these compound could disrupt the peroxidation of polyunsaturated fatty acids, as well as other cell membrane-associated compounds. Carotenoid levels varied among millet varieties such as finger, little, foxtail and proso millets, with mean values of 1.99 mg/kg, 0.78 mg/kg, 1.73 mg/kg, and 3.66 mg/kg, respectively. Several carotenoids, such as β-carotene, cryptoxanthin, trans- or cis-lutein and zeaxanthin were found in cereals. In addition, major carotenoids in millet included lutein and zeaxanthin, while trace amounts of β-carotene were also present. Tocopherols and tocotrienols formed part of the vitamin E group of compounds. While naturally occurring vitamin E consisted of eight vitamers: namely, α-, β-, γ-, and δ-tocopherols including four corresponding unsaturated tocotrienols. Furthermore, α- and γ- tocopherols were the major vitamin E components, and the total tocopherol content in millet varieties was found to be in the range of 1.3–4.0 mg/100 g.
The effect of processing parameters

Millet requires dehusking before consumption and the degree of milling influence the nutritional value and revenue derived from millet sales. The pericarp, seed coat, and aleurone layer of cereal grains are particularly rich in polyphenols and phytates. Therefore, the phenolic antioxidant activity among of the various millet components could be described in the following decreasing order hull > whole grains > dehulled grains. The effect of dehulling on the antioxidant activity and total phenolic content was found to be affected by the specific variety as shown by Chandrasekara et al. Kumar et al. observed that the phytic acid and polyphenols in endospermic fractions of foxtail millet were significantly decreased, which resulted in a lower level of ferrous reducing and total antioxidant activity.

The millet was initially subjected to a wide range of thermal and hydrothermal treatments to render it fit for consumption. The antioxidant activity of millet might be modified by thermal treatment, based on the severity of heat treatment, duration of exposure and variety of the millet. The antioxidant activity of roasted millet was higher compared to the steamed grain sample, which could be attributed to an increase in the extractability of bound phenolics by the thermal degradation of the cellular constituents. Both steam and microwave treatments decreased the phenolic content of barnyard millet but were responsible for higher phenolic content in foxtail and proso millet. On the other hand, steam treatment resulted in lower flavonoid levels in all three types of millet, while microwave treatment caused a decrease in the flavonoid contents of foxtail millet and no significant change was noted in the barnyard and proso millets. Irradiation presented a non-thermal processing technology, and the γ-irradiation process increased the antioxidant properties of millet by improving the antioxidant enzymes. Extrusion is considered a versatile, cost-effective method of generating ready-to-eat grains and cereals. Lower moisture levels and high screw speeds generally yielded more desirable products from proso millet the exhibited greater expansion and higher antioxidant activity.

Germination or malting could activate dormant enzymes that triggered complex biochemical reactions, as well as significantly altered the biochemical and antioxidant features of millet products. With germinated millet as raw material, the antioxidant activity was the highest (91.34%) after a soaking period of 13.81 h and germination at 38.75°C for 35.82 h, which was the optimal conditions for flour production. An increase in the total antioxidant activity (29.0–45.23 mgAAE/g) and the reducing potential (0.53–0.76 μg/mL) following the germination of foxtail millet could be ascribed to high levels of phthalic acid, hex-3yl-ester, and hexadecenoic acid methylester. However, increases in DPPH (48.32%–59.62%) and hydrogen peroxide scavenging potential (35.44–63.07 mM = Trolox/g) were caused by higher levels of hexadecanoic acid methyl ester, 9,12-octadecadienoic acid ethyl ester and the synthesis of novel compounds including pentadecanoic acid and 14-methyl-methylester. An increase in the metal chelating capacity (34.92 mgEDTA/g – 57.38 mgEDTA/g) and in vitro antioxidant activity might be due to a general rise in phenolic and flavonoid content. However, Hejazi et al. (2016) found that germination slightly decreased the phenol content, DPPH, and ABTS activity of finger millet by as much as 25%. Malting induced dynamic alterations in the phenolic acid content of finger millet and affected its antioxidant activity. A three-fold in the level of protocatechuic acid was detected after malting for 96 h. However, an increase was evident in the content of the free phenolic acids, such as coumaric, ferulic, gallic, and vanillic acids. The content of bound phenolic acids such as ferulic, caffeic, and coumaric acids decreased 2-fold after 96 h of malting. The antioxidant activity coefficient increased in the presence of free phenolic acids, while a decline was induced by bound phenolic acids after malting for 96 h. Furthermore, heat treatment could affect the antioxidant properties of millet in various ways. Toasting resulted in an increase in the antioxidant properties of pearl millet, and decreased after cooking.

Fermented millet has been used in various ways in Asia and Africa, such as in vinegar and thin fermented porridge. Fermentation increased the levels of soluble phenolic compounds, condensed tannins, and individual phenolic compounds, while decreasing the levels of the bound compounds. Vinegar is a product of cereal fermentation that consists of bound phenolic acids and traces of free phenolic
acids. Fermentation induced the release of these bound phenolic acids via microbial catalysis, as well as alterations in the free phenolic acids into various other compounds.\(^{[77]}\) Moreover, microorganisms were also utilized in millet fermentation. Amadou et al.\(^{[38]}\) described the use of *Lactobacillus paracasei* Fn032, and Salar et al.\(^{[80]}\) delineated the use of *Aspergillus sojae*.\(^{[38,80]}\) Increases in the scavenging ability of DPPH radicals and reducing power were observed. Digestive enzyme treatment also increased antioxidant activity by releasing more antioxidant components such as polyphenols from the millets.\(^{[29,30,81]}\)

**Challenges and future perspectives**

From the literature reviewed above, it was evident that millet possessed various substances with antioxidant properties, and these ingredients were increased by processing techniques such as germination, malting, and fermentation. Millet is an essential food source in poverty-stricken areas and is mainly used as feed and forage in North America and Europe. Moreover, a lack of innovative processing technologies inhibited the production of more convenient types of millet products. Providing healthier millet could be crucial in therapeutic dietary modification and promoting the utilization of millet food products. In addition, it is necessary for future research to establish additional scientific methods to evaluate the nutritional value of millet, especially the potential health benefits of antioxidants for animal and human models.

**Conclusion**

Oxidative stress was one of the causes of aging and diseases, and the risk of chronic diseases could be alleviated by the intake of antioxidants. Millet was the primary food source for the poorest people unable to afford expensive food products containing antioxidants. Millet not only provided high-quality nutrients, but also possessed antioxidant properties such as phenolic acids, flavonoids, tannins, and carbohydrates (e.g., XOs and insoluble fibers, specific proteins, and peptides, as well as certain micronutrients such as vitamin E and carotenoids). Furthermore, millet could be enriched with antioxidants (i.e., phenolics and flavonoid) through germination and fermentation. Wet thermal treatment tended to reduce the antioxidant activity of millet, while it was increased by dry thermal treatment due to the release of phenolic components.

**Acknowledgments**

This research was supported by the National Natural Science Foundation of China (No. 31801605); Projects of Beijing Technology and Business University Youth Fund (No. QNJJ2017-06); Projects of Beijing Municipal Science and Technology Project (Grant No. D17110500190000); National Agricultural Products Quality and Safety Risk Assessment of Major Projects (GJFP201801501); National Agricultural Products Quality and Safety Risk Assessment of Major Projects (GJFP201801504); The Agricultural Science and Technology Innovation Program (Y2018PT34).

**Funding**

This work was supported by the Projects of Beijing Municipal Science and Technology Project [D17110500190000]; National Agricultural Products Quality and Safety Risk Assessment of Major Projects [GJFP201801501, GJFP201801504]; Projects of Beijing Technology and Business University Youth Fund [QNJJ2017-06]; National Natural Science Foundation of China [31801605]; The Agricultural Science and Technology Innovation Program [Y2018PT34];

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