Pseudocereal grains: Nutritional value, health benefits and current applications for the development of gluten-free foods

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Abbreviations: DIAAS, digestible indispensable amino acid scores; dw, dry weight; FDA, Food and Drug Administration; GF, gluten free; ICR, Institute of Cancer Research; IGF-1, insulin like growth factor; IL, interleukin; LDL, low density lipoproteins; LPS, lipopolysaccharide; NO, nitric oxide; PDCAAS, protein digestibility corrected amino acid score; POx, prolin oxidase; PUFAs, polyunsaturated fatty acids; RS, resistant starch; TNF-α, tumor necrosis factor-alpha.

1. Introduction

Pseudocereals grains are edible seeds belonging to dicotyledonous species that are known as such due to their similar physical appearance and high starch content similar to true cereals (monocotyledonous of the *Poaceae* family) (Alvarez-Jubete et al., 2010a). Pseudocereals are promising crops of future due to their high genetic variability that is advantageous for them to be adapted to different environments from tropical to temperate climatic conditions (Joshi et al., 2018 and 2019; Ruiz et al., 2013). The most important species are quinoa (*Chenopodium quinoa* Willd), amaranth (*Amaranthus* sp.) and buckwheat (*Fagopyrum* sp.). Quinoa and amaranth belong to *Chenopodiaceae* family originated from the Andean region in South America. The genus *Fagopyrum* belongs to the *Polygonaceae* family and includes three cultivated species, *F. sculentum* (common buckwheat), *F. tataricum* (tartary buckwheat) and *F. cymosum* (tall buckwheat) that are native to Central and Western China.

Pseudocereals are a current trend in human diets as they are gluten-free (GF) grains and have an excellent nutritional and nutraceutical value. Moreover, recent research has pointed out the potential health benefits of pseudocereals depicting these crops as important resources for functional food development (Joshi et al., 2018 and 2019). This review is focused on the nutritional quality and bioactive compounds of the main pseudocereal grains (quinoa, amaranth and buckwheat), current *in vivo* evidence on their health benefits and development of GF products.

2. Nutritional value of pseudocereals

Pseudocereals have been described as “the grains of the twenty-first century” due to their excellent nutritional value (FAO, 2011) that is presented in Table 1. They are rich in starch, fiber and proteins of high quality with a balanced essential amino acid composition characterized by abundant amounts of sulfur- rich amino acids. They are also a good source of minerals (calcium, iron and zinc), vitamins, and phytochemicals such as saponins, polyphenols, phytosterols, phytosteroids, and betalains with potential health benefits.

1. Carbohydrates

Carbohydrates are the major nutritional components of pseudocereal grains that fluctuate between 60 and 80% of the seed dry weight (dw) (Joshi et al., 2018, 2019; Shukla et al., 2018). Starch is the main carbohydrate ranging from 58.1-64.2%, 65.0-75.0%, 54.5-54.4% of total seed dry weight in quinoa, amaranth and buckwheat, respectively (Repo-Carrasco-Valencia and Arana, 2017). Buckwheat starch has a relatively higher amylose content (18.3-47% of total starch) compared to quinoa (11-12% of total starch) and amaranth (7.8-34.3% of total starch) (Repo-Carrasco-Valencia and Arana, 2017).

The starch can be classified as rapidly digestible, slowly digestible or resistant, depending on how easily it is broken down in the gut (Lockyer and Nugent, 2017). Resistant starch (RS) is considered to provide health benefits as it cannot be digested and absorbed in the small intestine reaching the colon where it is slowly fermented by microorganisms to produce short-chain fatty acids (Lehmann and Robin, 2007). To provide health benefits, current dietary guidelines suggest that starchy foods should contain at least 14% of RS on a total starch basis (EFSA, 2011). Among pseudocereals, common and tartary buckwheat have the highest levels of resistant starch (27-33.5%) (Skrabanja et al., 1998; Zhou et al., 2019a). The high levels of RS observed in buckwheat were proven: 1) to modulate blood glucose and lipid levels, 2) to regulate intestinal microbiota, and 3) to reduce obesity (Zhou et al., 2019a).

Pseudocereals are also an excellent source of dietary fiber. Total fiber content varies from 7.0 to 26.5% for quinoa, 2.7 to 17.3% for amaranth and 17.8% for buckwheat, which is in the same range as common cereal grains (Joshi et al., 2019 and 2018; Lamothe et al., 2015). Dietary fiber in quinoa and amaranth is mostly made up of insoluble polysaccharides

(78% of the total dietary fibre content) including homogalacturonans and rhamnogalacturonan-I with arabinan sidechains (55-60%), as well as highly branched xyloglucans (30%) and cellulose (Lamothe et al., 2015). On the other hand, 22% of total dietary fibre in quinoa and amaranth are soluble polysaccharides composed of xyloglucans (40-60% of the soluble fiber fraction) and arabinose-rich pectic polysaccharides (34-55% of soluble fiber fraction). Buckwheat grains contain a higher fibre content (17.8%) than other pseudocereals although with a lower soluble to insoluble dietary fiber ratio (0.5-0.28) (Dziedzic et al. 2012). Buckwheat dietary fiber is composed by 6.79% of lignin, 2.22% of hemicellulose and 10.64% of cellulose (Dziedzic et al. 2012).

Mono- and disaccharides are minor compounds of the carbohydrate fraction of pseudocereal grains. Glucose, fructose, arabinose and xylose are the main monosaccharides found in pseudocereals while sucrose and maltose are the most representative disaccharides. Simple carbohydrates content is relatively higher in quinoa and amaranth (3-5%) while it is notably lower in buckwheat (0.8%), compared to cereals (1-2%) (D´Amico et al., 2017; Pereira et al., 2019).

2.2. Protein

As in all plants, protein content and profile depend on genotype and growing conditions. Compared to cereals, pseudocereals have superior nutritional value mainly related to their higher protein levels that vary from 9.1-16.7% for quinoa, 13.1-21.5% for amaranth, and 5.7-14.2% for buckwheat (Joshi et al., 2019 and 2018; Nowak et al., 2016; Pereira et al., 2019; Shukla et al., 2018; Thanh-Tien et al., 2018), making them key contributors to human protein intake.

The major protein fractions of quinoa are 11S-type globulins and 2S albumins that account for 27.9-60.2% and 13.2-42.3% of total seed proteins, respectively, followed by glutelins (18.1-31.6). Prolamins represent a smaller protein fraction (0.5-19.3% of total seed protein) (Dakhili et al., 2019; D'Ámico et al., 2017). Amaranth proteins comprised of about 40% albumins, 20% globulins, 25-30% glutelins, and 2-3% prolamins (Bucaro et al., 2002; Venskutonis and Kraujalis, 2013). The main protein components of buckwheat seeds are 8S globulin and 13S globulins. Moreover, 2S globulins, glutelins and prolamins are minor protein fractions of common and tartary buckwheat (Zhou et al., 2019b).

When examining the quality of plant-based protein sources, the protein amino acid composition and bioavailability are important factors to consider. Pseudocereals have an exceptional balance of amino acids, with higher content of lysine, methionine and cysteine than common cereals primarily deficient in lysine and secondarily deficient in threonine and tryptophan (Motta et al., 2019). The recent discovery of seven new vicilin-like globulins containing 7.5% or more of lysine mass explains the high content of this amino acid in quinoa grain (Burrieza et al., 2019).

The quality of protein should not only consider the amino acid composition, but also the digestibility and the absorption of the produced hydrolysis products in the human gastrointestinal tract (Joye, 2019). The protein digestibility corrected amino acid score (PDCAAS) is widely use as indicator of the nutritional quality of proteins. PDCAAS for proteins from different amaranth cultivars fluctuated between 23.7-36.2%, suggesting that this pseudocereal could be adequate as a complementary protein source (Aguilar et al., 2015). True ileal digestibility values of amino acids determined *in vivo* are often used for the characterization of protein quality in different foods and acquisition of digestible indispensable amino acid scores (DIAAS) in adult humans. According to DIAAS, common (68) and tartary (47) buckwheat are better protein sources for human consumption than oat (43), wheat (20), brown rice (42), polished rice (37), prosso millet (7), foxtail millet (10) and adlay (13) (Han et al. 2019a).

2.3. Lipids

Lipid content of quinoa (4.0-7.6%), amaranth (5.6-10.9%) and buckwheat (0.75-7.4%) is much higher than that of other cereals (Joshi et al., 2018; Shukla et al., 2018; Tang et al., 2016). The fatty acid profile is important for evaluating the nutritional value of the oil, especially the content of polyunsaturated fatty acids (PUFAs). Unsaturated fatty acids are the predominant fatty acids in quinoa (71-84.5% of total lipids), amaranth (61.0-87.3% of total lipids) and buckwheat (80.1-80.9% of total lipids), therefore, oil from this pseudocereals is considered of good nutritional quality. Majority of amaranth and quinoa accessions has more than 70-80% of total fatty acids represented by linoleic (C18:2, ω-6) and α-linolenic acids (C18:3, ω-3) (Pachari-Vera et al., 2019; Tang et al., 2016) whereas oleic acid (C18:1) and linoleic acid are the major abundant fatty acids in buckwheat (Shukla et al., 2018). Oil from pseudocereals is also represented by a minor amount of saturated fatty acids. Total saturated fatty acids fluctuate between 15.5-29.0%, 20.1-30.9, and 18.8-19.5% of total lipids in quinoa, amaranth and buckwheat, respectively (Pereira et al., 2019; Thanh-Tien et al., 2018). Palmitic acid is the most abundant unsaturated fatty acid in all pseudocereals.

In terms of nutritional quality of lipid fraction, the ω-6/ ω-3 ratio has been recognized to be a more important criterion than the total content of fatty acids (Tang et al., 2016). There is increasing evidence pointing out that unbalanced ω-6/ω-3 ratio in favour of ω-6 PUFAs is highly prothrombotic and proinflammatory due to its contribution to the prevalence of atherosclerosis, obesity, and diabetes (Kromhout and de Goede, 2014; Simopoulos, 2008). In fact, regular consumption of diets rich in ω-3 PUFAs has been associated with low incidence of these diseases (Adler et al., 1994; Kromann and Green, 1980; Schraer et al., 1999). It is worth to note that considering the negative health impact of lipids with high ω-6/ω-3 ratio, quinoa oil (ω-6/ω-3 ratio: 4.7-19.6) has a better nutritional quality than amaranth oil (ω-6/ω-3 ratio: 33.0-68.9) (Paucar-Menacho et al., 2018).

2.4. Minerals and vitamins

Most of the mineral compounds present in pseudocereal grains are located in the bran, therefore, whole pseudocereals grains are good sources of minerals (Pongrac et al., 2013). Amaranth contains the highest amount of minerals followed by quinoa and buckwheat (Joshi et al., 2019; Marques-Coelho et al., 2018; Nowak et al., 2016; Zhang and Xu, 2017). In a comparative study, it was observed that tartary buckwheat seeds contained higher levels of minerals than common buckwheat (Bonafaccia et al., 2003). The main minerals in pseudocereals are potassium, phosphorous, and magnesium (Table 1). High calcium content in amaranth might be of special relevance to provide health benefits for celiac individuals considering they are more prone to osteopenia and osteoporosis (Chand and Mihas, 2006; Rodrigo, 2006).

The vitamin content of pseudocereals is also interesting, because they contain high levels of B vitamins. Quinoa seeds contain high levels of vitamin B_6 and folate, whose amounts can cover the requirements of children and adults (USDA, 2005). Likewise, riboflavin content of quinoa seeds contributes to 80% of the daily needs of children and 40% of adults (USDA, 2005). Recently, Rybicka and Gliszczynska-Swiglo (2017) compared the vitamin B profile of 14 gluten free flours including teff, oat, rice, millet, chickpea, chestnut, corn, acorn, buckwheat, and amaranth. In this study, buckwheat flours were identified as the best sources of vitamin B_2 and B_3 while amaranth showed the highest concentration of vitamin B_6. In addition, 100 g portion of amaranth was estimated to cover the 53% of recommended daily allowance in Poland, the US and Canada for vitamin B_6 (Rybicka and Gliszczynska-Swiglo, 2017). Vitamin E is the term to describe a group of 8 fat-soluble compounds: tocopherols (α, β, γ, and δ isomers) and tocotrienols (α, β, γ, and δ isomers), which exhibit antioxidant activity and are nutritionally essential. Out of the different vitamin E isomers, α-tocopherol has the highest vitamin E activity. The vitamin E activity is expressed α-tocopherol equivalents, which accounts for about 90% of the activity in human tissue; the relative potency of α-, β-,γ-, and δ-tocopherol is reported to be approximately 100:50:25:1. All four tocopherol isoforms as well as α- and β-tocotrienols has been detected in quinoa (Lin et al., 2019). Total tocopherol content of quinoa was reported to be between 37.5 and 77.7 mg/kg (Lin et al., 2019; Tang et al., 2016). Although quinoa has a concentration of total tocopherols similar to other commonly consumed cereal grains, individual tocopherol profile is different, with γ-tocopherol followed by α-tocopherol as the main components (Pachari-Vera et al., 2019; Tang et al. 2015, 2016). Total vitamin E concentration in amaranth seeds was reported 3-times lower compared to quinoa seeds (Tang et al., 2016). In terms of profiles of the different homologues, δ-tocopherol was the predominant compound in amaranth seeds (7.7 mg/kg on average), followed by β-, α- and γ-tocopherol (Tang et al., 2016). Common buckwheat contains the lowest content of vitamin E among pseudocereals (9.5-16.4 mg/kg) with a similar profile to quinoa, in which γ-tocopherol is the most abundant (4.8-49 mg/kg) followed by α-tocopherol (1.3-7.9 mg/kg), and δ-tocopherol (0.9-5.6 mg/kg) (Alvarez-Jubete et al., 2010a).

Carotenoids are a subfamily of terpenoids known for their physiological function as natural antioxidants as well as their ability to provide protection from UV radiation. In addition, some carotenoids such as α- and β-carotene are the precursors of vitamin A (Fiedor et al., 2014). Quinoa seeds have been reported to be a better source of carotenoids (4.6-18.1 mg/kg dw) than amaranth (3.7-4.7 mg/kg dw) and buckwheat seeds (3.6-3.7 mg/kg dw) (Tang et al., 2016; Tuan et al., 2013). Lutein is the main carotenoid present in quinoa, amaranth and buckwheat seeds (Tang et al., 2016, Tuan et al., 2013; Zhang et al., 2016).

3. Bioactive compounds

Bioactive compounds are non-nutrient plant components with health benefits that are only recently becoming recognized. The groups of bioactive components in pseudocereal grains include saponins, phenolic compounds, phytosterols, phytoecdysteroids, polysaccharides, betalains, bioactive proteins and peptides. This section will examine the structural diversity of each group of bioactive compounds in pseudocereals and will review recent evidences for their biological activity.

3.1. Triterpenoid saponins

Triterpenoid saponins consist of an aglycone, a pentacyclic C_{30} skeleton called sapogenin, linked to one or more oligosaccharide chains with a degree of polymerization from 2 to 5 hexoses or pentoses (Mroczek, 2015). Monodesmosidic saponins are typically composed of a single sugar chain attached at C-3. Bisdesmosidic saponins contain two sugar chains, one bound through an ether linkage at C-3 and other linked through ester linkage at C-28. Tridesmosidic saponins have three sugar chains and are seldom found.

Saponin profile of quinoa and amaranth seeds is highly diverse as demonstrated for several cultivars and species (Mroczek, 2015; Zehring et al., 2015). To date, a total of 87 and 15 different saponins have been identified in quinoa and amaranth seeds, respectively, located mainly in the bran (Mald et al., 2006; Mroczek, 2015). Most of saponins in both pseudocereals grains are bisdesmosidic although a tridesmosidic saponin has also been identified in *Amaranthus caudatus* (Rastrelli et al., 1998). Oligosaccharide chain (with up to four monosaccharide units) is commonly composed of glucose, galactose, arabinose, glucuronic acid or xylose, and linked to the aglycone at the carbon atom C-3, C-23 or C-28 (Lin et al., 2019).

Saponins are responsible for the bitter taste of quinoa seeds. Traditionally saponins are removed from quinoa seeds by steeping or washing the grains in water to improve their organoleptic properties prior to consumption. Due to this traditional method has some disadvantages including increased water waste and low efficiency, new quinoa varieties with low-saponin content have been developed by plant breeders. Therefore, total content of saponins in quinoa shows a high variability among genotypes. This variability in the saponin content has been also reported for amaranth seeds. Gas liquid chromatography coupled to mass spectrometry analysis allowed the quantification of the most abundant saponins in *Chenopodium quinoa* seeds that are composed of aglycones such as oleanolic acid, hederagenin, serjanic acid and phytolaccagenic acid (Gómez-Caravaca et al., 2011; Herrera et al., 2019). In fact, 3-O-α-L-arabinopyranosyl-(1f3)-β-D-glucuronopyranosyl serjanic acid 28-O-β-D-glucopyranosyl ester followed by 3-O-α-L-arabinopyranosyl phytolaccagenic acid 28-O-β-D-glucopyranosyl ester were determined as the main saponins in different quinoa varieties (Gómez-Caravaca et al., 2011). The highest saponins content has been reported for bitter quinoa varieties (470.0-1633.3 mg/100 g dw) followed by sweet quinoa varieties (20.0-990.6 mg/100 g dw) and amaranth seeds (0.1-0.5 mg/100 g dw) (Herrera et al., 2019; Mroczek, 2015; Oleszek et al., 1999). Triterpenoid saponins in amaranth grains contain oleanolic acid, hederagenin or phytolaccagenic acid as the most abundant aglycones (Junkuszew et al., 1998).

Biological activities of triterpenoid saponins from plant materials have been extensively reviewed (Lin et al. 2019; Mroczek et al., 2015). These compounds may display diverse health positive effects including antifungal, antiviral, antiproliferative, hepatoprotective, immunomodulatory, hypolipidemic, antidiabetic, antiosteoporosis, as well as anthelmintic actions (Mroczek et al., 2015). Moreover, hemolytic activity of saponins is the most studied adverse health effect. Intravenous administration of saponins causes increased erythrocyte permeability and release of hemoglobin. I*n vitro* studies have demonstrated that structural features and concentration of individual saponins are determinant either for positive or negative health effects (Mroczek et al., 2015; Zehring et al. 2015). To date, there are few investigations focused on the study of the bioactivity of crude extracts or isolated saponins from quinoa and amaranth seeds. Earlier studies have reported the antifungal activities of saponin fraction from quinoa seeds against *Candida albicans* (Woldemichael and Wink 2001). A further study demonstrated that crude saponin fraction from quinoa display anti-inflammatory activity in lipopolysaccharide (LPS) induced RAW 264.7 murine macrophages by inhibition of the production of nitric oxide (NO), tumor necrosis factor (TNF)-α, and interleukin (IL)-6 (Yao et al., 2014a). Quinoa saponins have also shown antiobesity effects *in vitro* by inhibition of triglyceride accumulation in adipocytes and suppression of adipogenesis in 3T3-L1 adipocytes (Yao et al., 2015). On the other hand, Zehring et al. (2015) observed that saponin extract from different amaranth varieties exhibited a weak hemolytic activity and only one compound out of more than ten saponins tested was hemolytic.

Unfortunately, bioavailability of saponins is low due to their low membrane permeability or the chemical transformations occurred during gastrointestinal digestion and colonic fermentation (Navarro del Hierro et al., 2018). Functional ingredients are being developed by the application of chemical, microbial and enzymatic hydrolysis to quinoa bran (Herrera et al., 2019). These treatments are aimed at the transformation of saponins to sapogenins, the latter having superior bioavailability and bioactivities than the former saponins (Choi et al., 2005). As reported, sapogenins from quinoa and amaranth also display biological effects. As example, Lozano et al. (2013) demonstrated that sapogenin extract from quinoa and isolated oleanolic acid, phytolaccagenic acid, hederagenine, and methyl oleanate waste exhibited anti-inflammatory activity *in vivo* using carrageenan-induced paw edema and croton-induced ear edema as murine models of acute inflammation (Lozano et al., 2013). Furthermore, antitumor activity of isolated quinoa sapogenins was demonstrated by Suxo et al. (2018) who observed that hederagenin was the most cytotoxic compound (IC_{50} = 27.3 μM) followed by methyl oleanate (IC_{50} = 80.3 μM), oleanolic (IC_{50} = 90.9 μM), and phyolaccagenic acids (IC_{50} = 105.1 μM) in the breast cancer cell line JIMT-1 (Suxo et al., 2018). The cytotoxic effect of oleanolic acid and derivatives has also been confirmed in other cancer cell lines including prostate cancer PC3 (IC_{50} = 6.5 μM), lung cancer A549 (IC_{50} = 0.4 μM), breast cancer MCF-7 (IC_{50} = 35.4 μM), and gastric cancer BGC823 (IC_{50} = 2.6 μM) (Hao et al., 2013).

3.2. Phenolic compounds

Over the last two decades, phenolic compounds have attracted much interest due to their health benefits and contribution to the prevention of chronic diseases (Okarter and Liu, 2010). The dietary phenolic compounds contribute to the maintenance of a healthy gut by modulating the gut microbial balance (beneficial bacteria/pathogen bacteria). The screening of different pseudocereal varieties with regard to the phenolic profile is a very promising tool in the GF food design in terms of improving the nutritional quality (Rochetti et al., 2019). Recently, metabolomic analysis of a variety of pseudocereal flours have revealed a wide diversity in flavonoids (anthocyanins, flavones, flavanones, isoflavonoids, flavonols, and flavanols), phenolic acids (belonging to hydroxycinnamics, hydroxybenzoics, and hydroxyphenylacetics), and tyrosol derivatives being the most represented (Rochetti et al., 2019). Table 2 illustrates the phenolic diversity of pseudocereals grains.

Phenolic compounds are present in pseudocereal grains in three forms: soluble free and soluble conjugated to sugars or other low molecular mass components, and insoluble bound forms. Most of quinoa phenolic compounds are in the free form ranging from 167.2 to 308.3 mg gallic acid equivalents/100 g dw (Han et al., 2019b). The contribution of the free fraction to the total phenolic content varied from 53.5% to 78.0% in seven quinoa varieties, gallic and ferulic acids being the dominant compounds (Han et al., 2019b). Flavonoids are the second most abundant phenolic group in quinoa seeds (Han et al., 2019b; Rochetti et al., 2019). Rutin, quercetin and kaempferol derivatives mostly present in the free fraction are the main flavonoids present in quinoa seeds. Bound phenolic compounds in quinoa are present in lower concentrations, ferulic acid being the most abundant phenolic compound of this fraction (Gómez-Caravaca et al., 2011; Han et al., 2019b; Rochetti et al., 2019; Tang et al., 2015). Black and red quinoa cultivars have been reported to be particularly high in anthocyanins such as cyanidin, cyanidin 3-*O*-glucosyl-rutinoside and cyanidin 3-*O*-sambubioside 5-*O*-glucoside (Rochetti et al., 2019). Anthocyanin rich grain varieties have been shown to reduce *in vitro* starch hydrolysis index due to their ability to inhibit carbohydrate-digesting enzymes, thus reducing the risk of type-2 diabetes and/or obesity (Han et al., 2019b). This aspect may be considered of great interest considering the design of GF foods with functional properties.

Among pseudocereals, amaranth present the lowest total phenolic content (21.2-57.0 mg gallic acid equivalents/100 g dw) (Alvarez-Jubete et al., 2010b; Rochetti et al., 2017, 2019), mainly composed of phenolic acids such as ferulic acid followed by flavonoids and other phenolic compounds such as sesamin, tyrosol and cardol (Rochetti et al., 2017, 2019). Quercetin 7,4'-*O*-diglucoside, quercetin 4'-*O*-glucoside and quercetin 3-*O*-glucoside are the most abundant flavonoids in non-pigmented varieties (Paucar-Menacho et al., 2018; Rochetti et al., 2017), and anthocyanins such as cyanidin 3-*O*-sambubioside 5-*O*-glucoside in pigmented amaranth varieties (Rochetti et al., 2019).

Buckwheat can be considered the best source of phenolic compounds among pseudocereals (275.5-532.0 mg gallic acid equivalents/100 g dw) (Liu et al., 2019; Rochetti et al., 2019). Buckwheat phenolic profile includes tyrosol (22.8 mg/100 g), alkylphenol (15.6 mg/100 g dw) and phenolic acids (227.7 mg/100 g dw) (Rochetti et al., 2019). Moreover, tartary buckwheat genotypes exhibit notably higher concentration of soluble phenolic compounds (667 mg gallic acid equivalents/g) than common buckwheat genotypes (Liu et al., 2019). Protocatechaldehyde, vanillic and caffeic acids in common buckwheat and salicylic acid in the tartary buckwheat are predominant phenolic acids in the soluble phenolic fraction (Liu et al., 2019). Nutraceutical properties of buckwheat have been mainly attributed to the presence of eight major flavonoids, rutin, orientin, quercetin, quercitrin, homoorientin, vitexin, and isovitexin (Zielinska et al., 2012). Tartary buckwheat groats have shown higher concentration of rutin (8.1 mg/g) than common buckwheat groats (0.2 mg/g) (Wijngaard and Arendt, 2006). Moreover, buckwheat seeds is the only pseudocereal that has been reported to contain proanthocyanidins so far (Zhu, 2019), being the majority of these compounds soluble (>70%) (Verardo et al. 2010). Genetic diversity in proanthocyanidins of diverse buckwheat species has been reported (Ölschläger et al., 2008). The proanthocyanidins concentrations in common whole buckwheat grains ranged from 15.4-40.6 mg/100 g dw (Ölschläger et al. 2008). The identified proanthocyanins in common and tartary buckwheat seeds are included in Table 2. It was reported that catechin (0.6-66.0 mg/100 g), epicatechin (2.3-11.0 mg/100 g) and epiafzelechin-(4-8)-epicatechin-(3,4-dimethyl)-gallate (1.7-5.7 mg/100 g) are the predominant compounds of this phenolic group (Ölschläger et al. 2008).

3.3. Phytosterols

Phytosterols are members of the triterpenoid family with similar structure to cholesterol found in animals and humans. The chemical structure comprises a 27-30-carbon ring with hydroxyl groups (Moreau et al., 2018). Phytosterols are found in four different forms: free sterols, steryl esters of fatty and phenolic acids, steryl glycosides, and acylated steryl glycosides. Free phytosterols contain a double bond in the B-ring between C-5 and C-6, or C7 and C-8, also known as Δ^5 and Δ^7-sterols, respectively. While quinoa and buckwheat are rich in Δ^5-sterols, amaranth species contain predominantly Δ^7-sterols (Moreau et al., 2018). Main Δ^5-sterols identified in quinoa and buckwheat include β-sitosterol, campesterol and stigmasterol, the former compound reaching 60-70% of total phytosterol content (Gornas et al., 2016; Guo et al., 2012; Islam et al., 2017). A series of minor phytosterols have also been identified in buckwheat oil represented by avenasterol, Δ^7-stigmasterol, cycloartenol, 24-methylenecyclo-artanol, and Δ^7-avenasterol (Dziedzic et al., 2016; Gornas et al., 2016). In contrast, phytosterols in amaranth grains are dominated by spinasterol (73.7%), followed by minor proportions of Δ^7-stigmasterol (11.9%), Δ^7-campesterol (3.5%), Δ^7-avenasterol (2.1%), Δ^5-campesterol (1.4%), and Δ^5-stigmasterol (0.9%) (Münger et al., 2015). Total phytosterol content in quinoa (38.8-41.2 mg/100 g) is relatively lower compared to buckwheat (69-96 mg/100 g) and amaranth (104.5 mg/100 g) grains (Islam et al., 2017; Dziedzic et al., 2016). Regarding health benefits, several countries have approved health claims for phytosterols and reduced blood LDL cholesterol (Moreau et al., 2018). In addition, Rideout et al. (2015) analyzed data from 12 randomized control trials and confirmed the triglyceride lowering effect of phytosterols in healthy and hypertriglyceridemic patients, being this effect greater in the latter patients group.

3.4. Phytoecdysteroids

Phytoecdysteroids are secondary metabolites which protect plants against nematodes and insects (Dinan and Lafont, 2009). These compounds are polyhydroxylated containing a cyclopentano-perhydrophenanthrene ring system. Chemical structure of phytoecdysteroids in plants is highly diverse (Bajguz et al., 2015). These compounds are concentrated in the bran and mainly present as free and polar/apolar conjugated forms, being classified as C27- and

C28-phytoecdysteroids based on the number of carbon atoms in their molecules (Kumpun et al., 2011).

It is known that quinoa is the only pseudocereal that contains phytoecdysteroids (138-570 µg/g) (Graf et al., 2016). A total of 36 compounds of this group have been identified (Lin et al., 2019) being C-27 phytoecdysteroids the most abundant in quinoa, particularly the 20-hydroxyecdysone (20E, 184-497 µg/g dw) (Graf et al., 2015). C28-phytoecdysteroids such as makisterone A, 24-epi-makisterone A, and 24(28)-dehydromakisterone A are the second most abundant group of steroids in quinoa seeds (Graf et al., 2015).

The range of beneficial health effects of quinoa phytoecdysteroids is very wide (Lin et al., 2019). These compounds have been proposed as promising bioactives for protecting against skin aging due to their ability to scavenge free radicals, chelate metal ions and inhibit the activity of calf skin collagenases (Nsimba et al., 2008). Another area of interest of phytoecdysteroids is their use as safe replacement for anabolic steroids (Bathori et al., 2008). 20E may improve physical performance by promoting skeletal muscle synthesis as demonstrated in mammalian models (Gorelick-Feldman et al., 2008). More recently, i*n vivo* studies have shown that quinoa phytoecdysteroids display antiobesity effects. Foucault et al. (2014) demonstrated that supplementation of either quinoa extract or pure 20E protects against diet-induced obesity in mice fed a high-fat diet. In this study, the significant reduction in fat mass was attributed to an increased carbohydrate oxidation and lipid fecal excretion. Xia et al., (2014) showed that 20E has a protective role in counteracting memory deficits in rats with diabetes possibly through enhancing the antioxidative ability in the brain.

3.5. Carbohydrates

In the last years, plant polysaccharides have gained interest because of their multifunctionality and their low toxicity and few side-effects. Plant polysaccharides have demonstrated to regulate immunity, and to have anti-virus, anti-tumor and antioxidant activity (Chen & Huang, 2016), being these activities dependent on different structural parameters such as sugar composition, molecular weight, type of glycosidic bond, and degree of sulfation (Yao et al., 2014b). Because of the multifunctionality of polysaccharides, they are widely applied as food additives, and ingredients in functional foods, pharmaceutical products, and adjunct therapies (Fan et al., 2019). Among plant sources of polysaccharides, pseudocereals have become one of the most studied. Cordeiro et al. (2012) demonstrated the anti-ulcer properties in an ethanol-induced gastric damage rat model of arabinan and arabinan-rich pectic polysaccharides present in quinoa seeds. Four polysaccharide sub-fractions isolated and purified from quinoa showed significant radical scavenging and inmunomodulatory capacity (Yao et al., 2014b). A novel quinoa polysaccharide constituted by galacturonic acid and glucose monosaccharides was reported to exhibit radical scavenging effects, macrophages RAW264.7 proliferation promoting properties, suppressing activity of NO production, and cytotoxic activity against human liver SMMC 7721 and breast cancer MCF-7 cells (Hu et al., 2017). Recently, a soluble non-starch polysaccharide fraction from *Chenopodium quinoa*, mainly composed of mannose, rhamnose, galacturonic acid, glucose, galactose, xylose and arabinose, was demonstrated to exert ameliorative effects on the improvement of anti-cyclophosphamide induced immunosuppression in the Institute of Cancer Research (ICR) mice (Fan et al., 2019). Polysaccharides in buckwheat have been reported to be the primary active components responsible for its various pharmacological activities. The hypolipidemic, antitumor, immunoregulatory, antioxidant, and neuroprotective effects of polysaccharides isolated from buckwheat have been widely studied. They displayed a dose-dependent ability to scavenge radicals and to inhibit formation of superoxide anion (Lee et al., 2014; Zhu et al., 2016). Their antioxidant mechanism *in vitro* has been mainly attributed to an improvement in the activity of antioxidant enzymes, which can protect biomolecules against the damage induced by free radicals (Xie et al., 2015). Lin and Lin (2016) reported that buckwheat polysaccharides did not directly affect the growth of human prostate cancer PC-3 cells but, instead, reduced their amplification by promoting release of anti-inflammatory biomarkers. Moreover, buckwheat polysaccharides facilitated the secretion of several cell factors, including TNF-α, NO, IL-2 and IL-1β in macrophages, and showed potential for the treatment of leukemia (Wu & Lee, 2011). Also, once digested and metabolized in the intestine, polysaccharides produce short chain fatty acids that induce mucosal immunity by conjugating with intestinal epithelial cells and which may lead to apoptosis of tumor cells when they enter the blood circulatory system (Wu and Lee, 2011).

Fagopyritols are mono-, di-, and trigalactosyl derivatives of D-*chiro*-inositol identified in seeds of common and tartary buckwheat (Steadman et al., 2000). In common buckwheat, they constitute 40% of soluble carbohydrate. The fagopyritols have been reported to show many activities, such as anti-oxidant, anti-inflammatory, and anti-diabetic (Fortis-Barrera et al., 2013; Hu et al., 2015). A recent study has demonstrated that fagopyritols from tartary buckwheat significantly suppressed increase of blood glucose, decreased lipid level, and ameliorated insulin resistance *in vivo* in an insulin-resistant mice model. In addition, these carbohydrates enhanced the glucose consumption in both normal and insulin resistant HepG2 cell (Wu et al., 2018).

Buckwheat also contains the iminosugar D-fagomine as a minor component that might contribute to the alleged health benefits of this pseudocereal. The functional effects of D-fagomine include a reduction of post-prandial blood glucose concentration, achieved through the inhibition of intestinal disaccharidases, and reduction in high-fat-diet-induced weight gain, low-grade inflammation and impaired glucose tolerance, probably all achieved by counteracting adverse changes in gut microbiota. Moreover, D-fagomine has been demonstrated to promote the diversity of gut microbiota by increasing populations of

Bacteroidetes in healthy rats while mitigating the age-related reduction in the populations of putatively beneficial *Lactobacillus* and *Bifidobacterium* bacteria (Hereu et al., 2019). Moreover, the ability of D-fagomine to counteract sucrose-induced steatosis and hypertension has been recently reported, presumably by reducing the postprandial liver fructose levels (Ramos-Romero et al., 2019).

3.6. Betalains

Betalains are nitrogen-containing aromatic indole derivatives synthesized from tyrosine and characteristic of plants belonging to the order *Caryophyllales*, to which *Amaranthaceae* crops belong. According to their chemical structure, they can be subdivided into violet betacyanins or yellow betaxanthins which combination makes the red and orange shades that coexist in nature with the pure violet and yellow colors (Gandía-Herrero & García-Carmona, 2013). Most betacyanins are referred to by their common names, usually derived from the names of plants where they are found. Thus, the betacyanin from plants of the genus *Amaranthus* is called amaranthine. Because of their color, betalain-containing extracts were approved as natural colorants in food products and pharmaceuticals by the European Union (additive E-162) and the Food and Drug Administration (FDA) (additive 73.40). Total betaxanthins and betacyanins were quantified in the leaves of four cultivars of vegetable amaranth from Bangladesh (Khanam & Oba, 2013). Although the amounts of both groups of pigments as well as the antioxidant activity were highly dependent on the cultivars, the antioxidant properties were related with the phenols and not with the betalains.

Quinoa is considered a promising crop for the extraction of betalains although the results on their content in different quinoa varieties are controversial. Abderrahim and coworkers (2015) determined betalain contents (expressed as the sum of betacyanins and betaxanthins) in the range between 0.15 and 6.10 mg/100 g in some Peruvian Altiplano’s red quinoa seeds while Repo-Carrasco-Valencia et al. (2010) did not detect the presence of betacyanins in coloured quinoa seeds from the same Peruvian region. Tang and coworkers (2015) reported that the betanin and isobetanin contents in black and red quinoa cultivated in Canada were similar to those found in beetroot, although quantitative data were not provided. In a recent study, novel betacyanins and betaxanthins were identified in 29 Peruvian quinoa varieties, correlating their presence with the high antioxidant and free radical scavenging activities measured in grain extracts (Escribano et al., 2017). These authors suggested that colored quinoa seeds might be interesting as a natural source of bioactive betalains. These compounds have gained popularity as ingredient of functional foods owing to their antioxidant, anticancer, anti-lipidemic and antimicrobial activities (Gengatharan et al., 2015). To stabilize these compounds, microencapsulation has been recently studied (Aguilar-Tuesta et al., 2018). Maltodextrin-microencapsulations containing betacyanin and low saponin concentration might confer unique health-promoting properties.

3.7. Bioactive proteins and peptides

In addition to their nutritional role, pseudocereal proteins have been found to exert biological properties by themselves and/or as source of bioactive peptides. Mendonca and others (2009) reported reduction of the total and low density lipoproteins (LDL)-cholesterol levels in plasma of hamsters fed amaranth protein isolates. One of the hypotheses suggested explaining the hypocholesterolemic effect was referred to the fiber content of amaranth, and possibly to the amino acid profile of its constituent proteins. Similarly, a quinoa protein-enriched fraction was found to inhibit the re-absorption of bile acids in the small intestine, and to control the cholesterol synthesis and catabolism, resulting in the modulation of total cholesterol levels in plasma and liver of mice (Takao et al., 2005). Moreover, Conforti and others (2005) reported the antidiabetic activity of two amaranth varieties through α-amylase inhibition. Recently, the potential of amaranth proteins as antithrombotic agent has also been described (Sabbione et al., 2016a, b, c).

In the last decade, pseudocereals proteins have demonstrated to be an important source of bioactive peptides. Thus, peptides from amaranth, buckwheat, and quinoa proteins have been identified in hydrolyzates, gastrointestinal digests and fermented products (Table 3). The multifunctional properties including antioxidant, anti-hypertensive, anti-inflammatory, anti-diabetic, and chemopreventive activities demonstrated for some of those peptides have made pseudocereals proteins gain importance as food ingredients for the prevention and/or management of chronic diseases related to oxidative stress, hypertension and/or diabetes. However, further studies focused on demonstrating the bioavailability and mechanisms of action of these peptides should be needed to confirm their *in vivo* effects.

4. Health benefits of pseudocereals in humans

Despite pseudocereals' composition and properties, scientific evidence supporting health claims in *in vivo* models is still limited and restricted to few animal studies and human trials (Table 4). Mainly, the intake of pseudocereals or their bioactive constituents have been associated to benefits against obesity, pre-diabetes and diabetes-related complications. Thus, amaranth protein based diets have been found to reduce food intake and body weight through reduction of rat plasma ghrelin levels and increase of postprandial leptin and cholecystokinin levels (Mithila & Khanum, 2015), and to modify microbiota composition in a diet-induced obesity mouse model (Olguín-Calderón et al., 2019). Moreover, amaranth protein improved glucose tolerance and increased plasma insulin in a streptozotocin-induced diabetes model (Soriano-Santos et al., 2015) while protein hydrolyzates exerted significant anti-hypertensive activity in spontaneously hypertensive rats (Fritz et al., 2011), and antithrombotic effects in Wistar rats (Sabbione et al., 2016c). Tartary buckwheat protein has been reported to exert hypocholesterolemic and anti-inflammatory activities in mice fed a high fat diet for 6 weeks, also modulating microbiota composition of the animals (Zhou et al., 2018). Quinoa intake was found to prevent hyperglycemia and reduce total cholesterol and LDL-cholesterol in Wistar rats (Pasko et al., 2010) and obese diabetic mice (Noratto et al., 2019). In the latest model, quinoa was also found to modulate inflammatory biomarkers and diminish hepatic steatosis and total cholesterol accumulation in the liver. Moreover, the health benefits of quinoa phytoecdysteroid-enriched extracts have been demonstrated in a diet-induced obesity mouse model in which the extracts reduced adipose tissue, decreased the expression of genes involved in lipid storage, and attenuated mRNA levels of inflammation and insulin resistance (Foucault et al., 2011). These authors also observed an increase of energy expenditure without affecting food intake and activity, and an increase of glucose oxidation and fecal lipid excretion without any change in stool amount (Foucault et al., 2014).

To date, a reduced number of human trials have been conducted to evaluate the benefits of pseudocereals consumption (Table 4). In 50-65 month old boys belonging to low-income Ecuadorian families, administration, twice a day, of 100 g quinoa significantly augmented the plasma insulin like growth factor (IGF-1) levels when compared to the control group (Ruales et al., 2002). Thus, quinoa-enriched baby food was described to provide sufficient protein and other essential nutritional elements capable to prevent child malnutrition. Moreover, supplementation of diet with quinoa has been demonstrated to modulate cardiovascular and metabolic parameters in both healthy (Farinazzi-Machado et al., 2012), overweight and obese individuals (De Carvalho et al., 2014; Li et al., 2018b; Navarro-Pérez et al., 2017). Similarly, both acute and chronic administration of buckwheat to diabetic subjects resulted in modulatory effects on metabolic and cardiovascular markers (Qiu et al., 2016; Stringer et al., 2013).

4. Suitability and application of pseudocereals for development of gluten-free (GF) foods

In recent years, development of novel GF foods is becoming one of the major trends in food industry. In fact, this market is forecasted to expand at a compound annual growth rate of 9.1% from 2019 to 2025 (https://www.grandviewresearch.com/industry-analysis/gluten-free-products-market).

The constant increase in global GF sector is driven by the increased incidence of gluten-related diseases such as celiac disease, non-celiac sensitivity and wheat allergy in developed countries (Foschia et al., 2016). However, the fast growth of GF market sales is not solely accounted by the higher prevalence of gluten-related disorders but also by the increased popularity of these foods among healthy consumers that voluntarily choose to follow a GF diet as a lifestyle choice (Prada et al., 2019; Xhakollari et al., 2019). Even though GF diet is known to alleviate symptoms in patients suffering gluten-related diseases, several studies have shown that the nutritional quality of many GF foods is poor, and usually, GF items present higher fat, sugar and sodium levels and lower protein, minerals and dietary fiber contents than their gluten-containing counterparts (Cornicelli et al., 2018; Lasa et al., 2017; Miranda et al., 2014).

The rising demands for GF foods are boosting the research studies aimed developing novel healthy GF foods nutritionally balanced. The improvement of bioactive, technological and sensory characteristics remains a challenge for elaborating new GF items. Traditionally, rice and corn have been the main ingredients for GF foodstufs. However, in recent years, pseudocereals have emerged as alternative ingredients in GF formulations since they are naturally GF grains with a high nutritional value and constitute a valuable source of a wide range of bioactive compounds as previously reviewed (see section 3). Since the pericarp of the quinoa grain contains high amount of bitter saponins, it is usually removed via mechanical abrasion or washing before the seeds are used (Vega-Galvez et al., 2010). Quinoa desaponification keeps the nutrient-rich embryo and endosperm intact (Montemurro et al., 2019). Processing of buckwheat to be used as ingredient in GF foods usually includes a dehulling step (Dziedzic et al., 2016), while the whole amaranth seed are usually ground into flour (Guardianelli et al., 2019) to be used in GF foods.

4.1. Evidences supporting the inclusion of pseudocereals in GF foods

Although it is well known that pseudocereals lack gluten proteins in their composition, the absence of immunotoxicity for celiac people must be confirmed in order to use them as ingredients for GF foods elaboration. However, the believed safety of pseudocereal consumption for celiac people is usually based in their taxonomical classification rather than in a direct assessment of their immunochemical reactivity.

From the taxonomical point of view, cereals are all monocotyledonous species classified in the *Poaceae* family, while pseudocereals are dicotyledonous plants belonging to different families: buckwheat (*Fagopyron esculentum*) is placed in the *Polygonaceae* family, quinoa (*Chenopodium quinoa*) is placed in the *Chenopodiaceae* and amaranth is placed in the *Amaranthaceae* (Rosentrater and Evers, 2018). This fact determines the different content of toxic prolamins for celiacs in cereals and pseudocereals. In cereals like wheat, barley and rye, prolamins constitute 30-40% of total protein content, while the major storage proteins in pseudocereals are albumins (2S) and globulins (7S, 11S, or 13S), that represent more than 50% of the total proteins, being prolamins very scarce (~8%) (Ballabio et al., 2011; Rajnincová et al., 2019; Taylor et al., 2016). The major amino acids in cereal prolamins are proline and glutamine (both involved in the pathogenesis of celiac disease), while high levels of aspartate/asparagine, arginine, serine, leucine and glycine are found in quinoa globulins, showing buckwheat globulins high levels of aspartate/asparagine, glycine and glutamate/glutamine. In amaranth globulins, all amino acids are present, and they particularly did not show high levels of any individual amino acid (Mir et al., 2018; Taylor et al., 2016). The different proportion or prolamins and chemical characteristics of pseudocerals storage proteins compared to toxic cereal prolamins suggest the lack of toxicity of pseudocerals for celiac individuals and support their use as ingredients in GF foods.

In order to verify the pseudocerals safety for the diet of celiac people suggested by taxonomical characteristics, several studies have examined, from the biochemical and molecular point of view, the protein pattern and immunochemical reactivity against gliadin antibodies or serum from celiac subjects of quinoa, amaranth and buckwheat. Berti et al. (2004) demonstrated that the immunoreactivity of quinoa proteins extracted in different solvents was very low against either commercial anti-gliadin antibodies or serum of a celiac person, and was comparable to that of proteins from GF flour. Calderón de la Barca et al. (2010) evaluated the content of gluten-like proteins in cookies and breads produced from raw and popped amaranth flours by a commercial kit and confirm that it was lower than 20 ppm, the maximum content allowed for GF foods. In a more recent study, the immunoreactivity of the alcohol soluble fraction from quinoa and amaranth was evaluated in intestinal T-cell lines, cultures of duodenal explants from HLA-DQ2$^+$ celiac patients, and HLA-DQ8 transgenic mice (Bergamo et al., 2011). Authors concluded that both pseudocereals did not show immune cross-reactivity towards wheat gliadin and, therefore, they can be safely consumed by celiac persons. Ballabio et al. (2011) evaluated the possible tolerance by celiac patients of 40 different amaranth varieties by SDS-PAGE, immunoblotting and ELISA. This study confirmed the low content of gluten-like proteins (<20 ppm) in amaranth grains and the scarce cross-reactivity of amaranth proteins versus IgA and IgG from celiac persons sera. The presence of celiac-toxic prolamin epitopes in 15 quinoa cultivars and the ability of these epitopes to activate immune responses in celiac subjects were also studied by Zevallos et al. (2012). Authors concluded that generally quinoa is safe for celiac people, but 2 cultivars (Ayacuchana and Pasankalla) exhibited toxic epitopes that activated gliadin-specific T-cell lines. Nevertheless, authors indicate that the potentially low number of toxic epitopes in quinoa could be clinically irrelevant, but *in vivo* studies are necessary to confirm these results. The suitability of 11 different quinoa varieties for celiac individuals was also assessed in an *in vitro* study, and the results indicated that none of the quinoa varieties studied presented protein bands with electrophoretic mobility comparable with those of wheat prolamins and confirm the low binding affinity of quinoa proteins to anti-gliadin antibodies and IgAs from celiac subjects (Peñas et al., 2014). Recently, it has been reported that buckwheat is composed mainly by albumins and glutamins with high content of essential amino acids that are not able to react against a polyclonal anti-gliadin antibody (Rajnincova et al., 2019).

The biochemical and molecular results obtained in these studies together with the taxonomical classification of pseudocereal suggest their low risk to trigger an adverse reaction in celiac subjects. However, controlled clinical trials are necessary to confirm these results and support the inclusion of pseudocereals in the diet of celiac persons.

4.2. Development of GF products based on pseudocereals

During last decade, there has been extensive research on the development of different GF foods from quinoa, amaranth and buckwheat, mainly bakery products (bread, cookies, cakes, muffins, etc), snacks, pasta, and beverages (Table 5).

4.2.1. GF breads

GF breads commercially available exhibit poor quality and lower acceptability than the regular products, since gluten is a key element that provides the technological attributes to bread. Usually GF breads exhibit bad gas retention during fermentation and, therefore, they are characterized by low volume, crumbly texture and crumb hardness (Burešová et al., 2017). GF breads do not show the rheological, textural and baking properties that are unique in gluten-containing breads (Naqash et al., 2017). In addition to technological problems, GF breads are lower in dietary fiber, protein, minerals and vitamins than the conventional breads (Conte et al., 2020; Föste et al., 2014).

In an attempt to improve nutritional and technological properties of GF breads, research efforts have been focused on the use of pseudocereals for bread production since they are nutrient-dense grains and have high content of albumins and globulins that might improve technological characteristics of derived doughs and breads. In this context, Alvarez-Jubete et al. (2009a) evaluated the replacement of potato starch with amaranth, buckwheat or quinoa flour in a GF bread formulation containing 50% potato starch and 50% rice flour (Table 5). The authors observed that these breads were characterized by a softer crumb due to the presence of natural emulsifiers in pseudocereal flours, and those produced with quinoa and buckwheat had higher volume than the control one. Moreover, the addition of pseudocereals in bread improved the fiber, protein, calcium, iron and vitamin E contents, as well as the phenolic levels and antioxidant activity in breads (Alvarez-Jubete et al., 2009a,b; 2010b). Another study demonstrated that the substitution of corn starch with 40% of buckwheat flour also improved the nutritional profile of a GF bread formula by increasing levels of magnesium, phosphorus and potassium, and its content of phenolic compounds and antioxidant potential (Wronkowska et al., 2010). Addition of 10-30% of husked buckwheat decreased starch retrodegradation, enhanced anti-staling properties and provides a pleasant flavor and taste in rice-based GF breads (Torbica et al., 2010). Sakač et al. (2011) observed that the fortification of breads with wholegrain buckwheat flour from 10% to 30% also increased strongly the antioxidant activity of rice-based GF breads. Wolter et al. (2013) reported that GF breads obtained from buckwheat or quinoa flours showed lower predicted glycemic index than breads containing 100% of wheat flour. The inclusion of up to 40% of dehulled buckwheat flour also improved the leavening properties of a commercial GF bread mixture (Mariotti et al., 2013). The authors demonstrated that the improvements of dough development by buckwheat addition can be attributed to an increased viscosity, due to its high dietary fiber levels, to the swelling and gelling characteristics of buckwheat starch and to the capacity of globulin protein fraction to form and stabilize emulsions. The inclusion of 5% of puffed buckwheat flour and 0.5% hydroxypropylmethylcellulose (HPMC) in bread resulted in a softer GF bread crumb. A more recent study demonstrated that breads produced with 100% buckwheat flour exhibited significantly higher mineral content (iron, zinc, calcium potassium, phosphorus, magnesium and cupper) than control wheat breads, but buckwheat brought about breads with lower specific volume and higher hardness than the control formulation (Sayed et al., 2016).

The addition of amaranth flour has been demonstrated to be a promising approach to obtain GF breads with high technological quality. In this sense, the elaboration of GF breads with 100% amaranth flour (60-70% of popped amaranth flour and 30-40% of raw amaranth flour) brought about loaves with homogeneous crumb and higher specific volume than GF control bread (Calderón de la Barca et al., 2010).

Results of another study indicated that the replacement of rice and corn flour with 10% of quinoa bran notably improved not only the bread volume by 7% but also the protein content by 17% in the final bread (Föste, et al., 2014). Breads containing 10% of quinoa bran also exhibited higher carbon dioxide formation and good appearance without compromising the taste. Similarly, the replacement of 40-100% of rice and corn flours by quinoa white flour had a positive impact on the specific volume of bread (that increased 33%) and also produced a homogeneous and finely distribution of gas bubbles in the crumb, without produce a bad impact in taste (Elgeti et al., 2014). Machado Alencar et al. (2015) also found that the replacement of a mixture containing rice flour, potato starch, cassava starch and sour tapioca starch with 20% of amaranth or quinoa whole flours improved the nutritional value of breads by increasing the levels of protein, fat and minerals and resulted in breads with specific volume and firmness similar to control bread. In addition, promising results were observed after inclusion of 25% quinoa in a GF bread formulation containing rice flour, potato starch and buckwheat flour. These breads presented softer texture and higher sensory scores than the control bread formulation (Turkut et al., 2016). A recent study has demonstrated that flatbreads containing also peanut oilcake and broccoli or beets instead broccoli exhibited higher acceptance, protein (>25% of protein) and mineral contents than wheat-based counterparts (Kahlon et al., 2019).

4.2.2. GF baking products (other than bread)

Cookies represent the largest category of snacks in bakery industry, and constitute the easiest baked product to formulate without gluten. Although the role of gluten in biscuit quality is not well defined, the lack of gluten provides biscuit with poorer technological and sensory properties (Di Cairano et al., 2018). In the last years, a growing number of studies have evaluated the addition of pseudocereals in the development of cookies with high nutritional, sensory and technological quality (Table 5).

Buckwheat has been largely used for producing tasty GF cookies. Buckwheat was incorporated at different levels (10%, 20%, and 30%) in rice-based cookie formulations, and it was found that the addition of 10-20% buckwheat produced cookies with the highest sensory scores in terms of flavor, rupture and chewiness (Torbica et al., 2012). Kaur et al., (2015) prepared GF biscuits from buckwheat flour incorporating various gums and observed than those contained buckwheat flour and xanthan gum exhibited good color, appearance, flavor and overall acceptability and decreased fracture strength compared to wheat-based cookies. In a recent study, an addition of 30% of tartary buckwheat malt in rice-based cookies provided higher protein, fiber, resistant starch, total phenolic and quercetin contents, as well as higher antioxidant activity and lower glycemic index than control cookies containing the same proportion of raw tartary buckwheat (Molinari et al., 2018). This study highlights the beneficial impact of incorporating germinated pseudocereals to improve the nutritional and functional quality of GF cookies.

The suitability of quinoa to obtain cookies with good quality has also been demonstrated by Brito et al. (2015), who optimized cookie formulation (30% quinoa flour, 25% quinoa flakes and 45% corn starch) to obtain cookies rich in dietary fibre, essential amino acids, linolenic acid and minerals. These cookies exhibited good sensory acceptability. More recently, Jan et al. (2018) optimized the process parameters for the elaboration of GF cookies from quinoa flour with antioxidant activity and satisfying quality in terms of color, spread factor, hardness and overall acceptability. The incorporation of amaranth (20% of popped amaranth flour and 13% of whole-grain popped amaranth) produced cookies with expansion factor and hardness similar to other GF cookies and high protein content (Calderón de la Barca et al., 2010). Germinated amaranth also showed great potential to produce GF cookies with acceptable quality and higher total dietary fiber and antioxidant activity than cookies developed from raw amaranth and wheat flours (Chauhan et al., 2015).

New trends in GF bakery products derived from pseudocereals are increasingly spreading to formulation of novel snacks. Bhaduri et al. (2013) evaluated the inclusion of quinoa flour in muffin formulations and concluded that the addition from 25% to 75% of quinoa flour in rice-based formulations brought about muffins with soft texture and good overall consumer acceptability. Likewise, it has been reported that the incorporation of 10% of quinoa flour to oat and rice flour improved the sensory attributes such as appearance, color texture, flavor and taste of GF products such as muffins, cookies, pies, cakes, and tarts compared to control products (Kaur and Kaur, 2017). Sedej et al. (2011) formulated GF crackers from refined and wholegrain buckwheat flour with acceptable taste and appearance and with higher content of tocopherol and total phenolics than control wheat crackers. Wafer sheet formulations containing rice-buckwheat flours, rice-corn flours or rice-chestnut flours at different ratios have been also produced (Mert et al., 2015). The authors concluded that snacks containing rice-buckwheat flours (60:40) had the closest value of consistency and flow behavior index, as well as hardness and fracturability to wheat-based control formulation. In another study, quinoa was used to obtain four different types of low fat and salt snacks with or without spices (cayenne pepper, ginger or turmeric) with acceptable crispiness and acceptability (acceptance between 70-92%), as well as good taste, aroma, color and water activity which contributed to better microbial stability (Kahlon et al., 2016).

4.2.3. GF pasta

The development of GF pasta is particularly challenging since GF flours strongly affects the technological and sensory properties of this foodstuff. The replacement of wheat flour by alternative flours can cause pasta break down, a high loss of solid content to water during cooking and an undesirable texture because its high adhesiveness (Bastos et al., 2016).

Numerous researchers have investigated the development of GF pasta from pseudocereal flours (Table 5). The use of pre-gelatinized flour made from cassava starch and cassava bagasse (70:30), native cassava starch and amaranth flour (10:60:30) allowed the production of a fiber-enriched GF pasta with adequate color, firmness, stickiness and similar nutritional quality than commercial wheat-containing counterparts (Fiorda et al., 2013a). The pasta showed better cooking quality and lower solids loss to the cooking water than commercial GF pasta, as well as optimum cooking time (3 min) characteristic of instant pasta (Fiorda et al., 2013b).

Bastos et al. (2016) produced fresh spaghetti from dried potato pulp, extruded potato pulp and amaranth flour (65:10:25) with adequate firmness, and better color, and cooking characteristics, higher yield and less loss of solids to water than commercial wheat-based spaghetti. A combination of amaranth, quinoa and rice flours (10:40:50) was successfully applied to elaborate a high quality GF pasta with acceptable sensory properties and good acceptability, although the sticky texture was not appreciated by untrained panelists (Makdoud & Rosentrater, 2017). The pregelatinization treatment of buckwheat flour by roasting, extrusion, boiling or microwave appears to have a beneficial effect on the physicochemical, morphological and functional properties of the flour, thus enhancing its use in the pasta formulation (Sun et al., 2018). Recently, a recipe for a GF tagliatelle from quinoa flour containing also lupine flour, vegetable proteins and proline oxidase (POx) enzyme has been optimized (Linares-García et al., 2019). The optimal formulation containing quinoa flour and lupine flour (70:30) in combination with rice protein (12%) and POx-enzyme (1%) showed satisfying quality and a good nutritional value with high protein and dietary fiber contents.

4.2.4. GF beverages

Pseudocereals have retained a lot of attention as raw material for fermented and functional beverages development in the last decade. Beer is one of the most consumed fermented beverages worldwide. Several studies have evaluated the use of quinoa, amaranth and buckwheat to produce high-quality GF beer-like products. De Meo et al. (2011) performed micro-malting experiments on pseudocereals in order to understand their brewing behavior. The authors used an alternative alkaline steeping method to increase the total soluble nitrogen and free amino nitrogen contents in these pseudocereals. These preliminary studies indicated that these pseudocereals are suitable grains for producing GF beers and that alkaline steeping is a useful process for optimizing malt quality. On the other hand, a top fermented beer was brewed from 100% buckwheat malt and it showed similar physico-chemical properties (pH, free amino nitrogen, fermentability and total alcohol) than wheat-based beer. Buckwheat beer also exhibited an acceptable sensory quality with regards to odor, purity of taste, mouthfeel, tingling and bitterness (Nic Phiarais et al., 2010). Similarly, Deželak et al. (2014) prepared bottom-fermented GF beer-like beverages from buckwheat and quinoa malts, and observed that both beers showed similar values of viscosity and pH. Buckwheat beer-like products exhibited fermentability values, wort pH, soluble protein content and levels of volatile compounds commonly associated with beer aroma comparable to barley beer, but quinoa beers showed different values. However, quinoa beers showed values of viscosity and beverage pH comparable to those found in barley beer. Quinoa and buckwheat beverages showed a good general acceptance, but it was better in the latter. Buckwheat beer-like beverage appears quite similar to barley product, whereas quinoa shows many unique properties, such as higher nutritive properties, nutty aroma and black colour. The authors concluded that both pseudocereals showed good potential for brewing processes.

Fermented beverages, different than beer-like products, have been also produced from pseudocereals. In this sense, symbiotic beverages were developed using aqueous extracts obtained from quinoa and soy at different proportions. Formulations containing higher proportion of quinoa (70-100%) showed higher viscosity and consistency, while that containing low quinoa concentration (30%) exhibited the least hysteresis, the best sensory acceptance and chemical composition and the highest intention to purchase from a group of 80 volunteers (Bianchi et al., 2014). Quinoa seed water extract was also used to replace 25-100% of buffalo skim milk to produce fermented beverages (El-Deeb et al., 2014). Authors concluded that quinoa addition at a ratio of 75-100% allowed the production of fermented beverage with acceptable flavor, texture, color and appearance and enhanced content of some amino acids and minerals. Ludena-Urquizo et al. (2017) produced a beverage from different quinoa varieties (Rosada de Huancayo and Pasankalla) fermented with lactic acid bacteria. Both varieties were suitable as raw materials for producing fermented beverages with high content of protein, fiber, vitamins and minerals but Pasankalla brought about fermented beverages with higher protein and lower saponin concentration and lower loss of viscosity during the fermentation process. Both drinks had viable and stable microbial load during the storage period (28 days).

6. Future perspectives

Quinoa, amaranth and buckwheat are traditionally important and environmental stress-tolerant pseudocereals that are gaining popularity among the consumers and scientific community around the world. Beyond their excellent nutritional profile, the absence of gluten in these pseudocereals has made them a good alternative for the development of palatable GF products that ensure an adequate intake of nutrients in subjects with celiac disease whose number is rising day by day. The variety of nutritionally balanced gluten-free products containing pseudocereals is suffering a fast growth in the last years. However, usually pseudocereal-based foostufs exhibit poorer texture and sensory properties than the gluten-containing counterparts. More work is needed in order to optimize gluten-free formulations to meet sensory and textural demands of modern consumers. Pseudocereals also contain a plethora of bioactive compounds including saponins, phenolic compounds, phytosterols, phytoecdysteroids, polysaccharides, and bioactive proteins and peptides. These compounds are considered the main responsible for the beneficial effects on different body systems linked to the consumption of pseudocereals, thus helping to promote human health and to reduce risk of different chronic disorders including cancer, cardiovascular diseases, diabetes, and aging. However, the available evidence on these health benefits is still limited, making needed further investigation, with a particular focus on elucidating the mechanism through which bioactive compounds operate to deliver these desirable outcomes. Other aspects, such as the bioavailability of phytochemicals and their interactions are also worthy of investigation. Moreover, rigorously controlled human studies aim at confirming the health benefits in human population would increase the value of pseudocereals as part of a balanced diet. Thus, there are still many opportunities to explore the nutrional and funcitional potential of pseudocereals.

Developing methods to improve pseudocereals varieties through various biotechnological, breeding and agronomic practices to address the productivity, yield and climate resilient is making substantial progress. Moreover, formulations and technological processes to improve pseudocereal nutritional and technofunctional properties without compromising the bioavailability of bioactive molecules are necessary to enhance their incorporation into GF foods. More related research in these topics would contribute to increase cultivation and commercial end-use of these grains in preference of conventional cereal grains in developed countries.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1. Nutritional composition of quinoa, amaranth and buckwheat grains

Nutritional composition	Quinoa	Amaranth	Buckwheat	References
Moisture (%, wet basis)	8.2-13.1	8.9-9.4	11.0	Alonso-Miravalles and O´Mahony (2018); Joshi et al. (2018, 2019); Nowak et al. (2016); Pereira et al. (2019)
Carbohydrates (% dry basis)	48.5-77.0	63.1-70.0	63.1-82.1	Alonso-Miravalles and O´Mahony (2018); Joshi et al. (2018); Nowak et al. (2016); Pereira et al. (2019)
Starch (% dry basis)	58.1-64.2	65.0-75.0	54.5-57.4	Alonso-Miravalles and O´Mahony (2018); Rochetti et al. (2019)
Total dietary fiber (% dry basis)	7.0-26.5	2.7-17.3	17.8	Alonso-Miravalles and O´Mahony (2018); Glorio et al. (2008); Joshi et al. (2018, 2019); Lamothe et al. (2015); Nowak et al. (2016)
Insoluble (% total fiber)	78.0	78.0-86.0	70.3	Lamothe et al. (2015); Zhu et al. (2014)
Soluble (% total fiber)	22.0	14.0-22.0	16.0	Lamothe et al. (2015); Zhu et al. (2014)
Crude protein (%)	9.1-16.7	13.1-21.5	5.7-14.2	Joshi et al. (2018, 2019); Nowak et al. (2016); Pereira et al. (2019); Shukla et al. (2018); Thanh-Tien et al. (2018)
Amino acid composition (g/ 100 g protein)				
Essential amino acids				
Threonine	2.1-8.9	3.3-5.0	3.9-4.0	Joshi et al. (2018); Nowak et al. (2016); Thanh-Tien et al. (2018); Venskutonis and Kraujalis (2013)
Valine	0.8-6.1	3.9-5.0	2.3-6.1	Joshi et al. (2018); Nowak et al. (2016); Shukla et al. (2018); Venskutonis and Kraujalis, (2013)
Phenylalanine	3.0-4.7	3.7-4.7	1.3-7.2	Joshi et al. (2018); Shukla et al. (2018); Venskutonis and Kraujalis, (2013)
Isoleucine	0.8-7.4	2.7-4.2	1.1-4.1	Joshi et al. (2018); Nowak et al. (2016); Shukla et al. (2018); Venskutonis and Kraujalis, (2013)
Leucine	2.3-9.4	4.2-6.9	2.2-7.6	Joshi et al. (2018); Nowak et al. (2016); Shukla et al. (2018); Venskutonis and Kraujalis, (2013)
Methionine	0.3-9.1	1.6-4.6	0.5-2.5	Joshi et al. (2018); Nowak et al. (2016); Shukla et al. (2018); Venskutonis and Kraujalis, (2013)
Tryptophan	0.6-1.9	0.9-1.8	1.83	Joshi et al. (2018); Nowak et al. (2016); Venskutonis and Kraujalis, (2013)
Lysine	2.4-7.8	4.8-8.00	4.2-8.6	Joshi et al. (2018); Nowak et al. (2016); Shukla et al. (2018); Venskutonis and Kraujalis (2013)
Non-essential amino acids				
Aspartate	8.0	7.3-10.7	7.6-16.6	Dakhili et al. (2019); Shukla et al. (2018); Venskutonis and Kraujalis (2013)
Glutamate	13.2	14.4-17.7	23.2-24.4	Dakhili et al. (2019); Thanh-Tien et al. (2018); Venskutonis and Kraujalis (2013)
Serine	3.4-5.7	4.9-9.3	3.2-8.6	Dakhili et al. (2019); D´Amico et al. (2017); Shukla et al. (2018); Venskutonis and Kraujalis, (2013);
Histidine	1.4-5.4	1.9-3.8	1.8-4.9	Dakhili et al. (2019); D´Amico et al. (2017); Thanh-Tien et al. (2018); Venskutonis and Kraujalis (2013)
Glycine	2.2-6.1	6.7-15.2	6.2-13.2	Dakhili et al. (2019); D´Amico et al. (2017); Shukla et al. (2018); Venskutonis and Kraujalis

				(2013)
Arginine	6.9-13.6	8.7-15.6	10-5-11.3	Dakhili et al. (2019); D´Amico et al. (2017); Thanh-Tien et al. (2018); Venskutonis and Kraujalis (2013)
Alanine	3.2-5.7	3.5-6.2	4.6-9.6	Dakhili et al. (2019); D´Amico et al. (2017); Shukla et al. (2018); Venskutonis and Kraujalis (2013)
Tyrosine	2.5-3.7	3.3-3.7	0.6-4.9	D´Amico et al. (2017); Shukla et al. (2018) Venskutonis and Kraujalis (2013)
Cysteine	0.1-2.7	2.1-3.6	0.8-3.5	Dakhili et al. (2019); Shukla et al. (2018); Venskutonis and Kraujalis (2013)
Proline	2.3-5.5	2.82-4.6	2.6-8.8	Dakhili et al. (2019); D´Amico et al. (2017); Shukla et al. (2018); Venskutonis and Kraujalis (2013)
Lipids (% dry basis)	4.0-7.6	5.6-10.9	0.7-7.4	Joshi et al. (2018, 2019); Nowak et al. (2016); Pereira et al. (2019); Shukla et al. (2018)
Saturated fatty acid (% lipids)	15.5-29.0	20.1-30.9	18.8-19.5	Pereira et al. (2019); Thanh-Tien et al. (2018)
Lauric (C12:0)	nd	nd	0.02-0-04	Thanh-Tien et al. (2018)
Myristic (C:14:0)	nd	nd	0.07-0.1	Thanh-Tien et al. (2018)
Pentadecylic (C15:0)	nd	nd	0.05-0.06	Thanh-Tien et al. (2018)
Palmitic (16:0)	9.3-10.7	18.8-20.2	13.2-18.5	Pachari-Vera et al. (2019); Shukla et al. (2018); Tang et al. (2016); Thanh-Tien et al. (2018)
Margaric (17:0)	nd	nd	0.05-0.06	Thanh-Tien et al. (2018)
Stearic (C18:0)	0.7-1.1	3.7-4.2	1.4-6.3	Tang et al. (2016); Thanh-Tien et al. (2018)
Arachidic (C20:0)	nd	nd	1.1-1.2	Thanh-Tien et al. (2018)
Behemic (C22:0)	nd	nd	1.1-1.3	Thanh-Tien et al. (2018)
Lignoceric (C24:0)	nd	nd	0.7-0.8	Thanh-Tien et al. (2018)
Unsaturated fatty acid (% lipids)	71.0-84.5	61.0-87.3	80.1-80.9	Kraujalis and Venskutonis (2013); Tang et al. (2016); Thanh-Tien et al. (2018)
Palmitoleic (16:1)	nd	nd	0.15-0.20	Thanh-Tien et al. (2018)
Oleic (18:1-9c)	15.7-31.1	22.7-31.8	35.7-47.9	Pachari-Vera et al. (2019); Shukla et al. (2018); Tang et al. (2016)
Vaccenic (18:1-11c)	1.3-1.7	1.4-2.00		Tang et al. (2016)
Linoleic (18:2 ω-6)	44.9-58.6	37.1-45.9	31.4-44.6	Pachari-Vera et al. (2019); Shukla et al. (2018); Tang et al. (2016)
Linolenic (18:3 ω-3)	3.0-11.1	0.6-1.4	0.0-5.3	Pachari-Vera et al. (2019); Shukla et al. (2018); Tang et al. (2016)
Gondoic (C20:1)	0.6-1.6	0.2-0.3	1.8-3.1	Tang et al. (2016); Thanh-Tien et al. (2018)
Erucic (C22:1)	nd-1.5	nd-0.1	0.2-0.5	Pachari-Vera et al. (2019); Shukla et al. (2018); Tang et al. (2016)
ω-6/ω-3	4.7-19.6	33.0-68.9	not reported	Pachari-Vera et al. (2019); Tang et al. (2016)
Mineral composition (mg/100 g, dry basis)				
Potassium	656-1475	290-434	450	Joshi et al. (2019); Marques-Coelho et al. (2018); Nowak et al. (2016); Zhang and Xu (2017)
Phosphorous	140-530	441-455	330-395.3	Joshi et al. (2019); Nowak et al. (2016); Marques-Coelho et al. (2018)
Calcium	27.5-148.7	175-206	46.5-50.4	Joshi et al. (2019); Marques-Coelho et al. (2018); Nowak et al. (2016); Zhang and Xu (2017)
Magnesium	207.0-502.0	254-266	390	Joshi et al. (2019); Nowak et al. (2016); Marques-Coelho et al. (2018)
Sodium	11.0-31.0	0.6	not reported	Marques-Coelho et al. (2018); Nowak et al. (2016)
Iron	1.1-16.7	12.0-17.4	11.8-14.9	Joshi et al. (2019); Marques-Coelho et al. (2018); Nowak et al. (2016); Zhang and Xu (2017)

Nutritional composition	**Quinoa**	**Amaranth**	**Buckwheat**	**References**
Zinc	0.8-4.8	3.7-5.20	2.1-2.4	Joshi et al. (2019); Marques-Coelho et al. (2018); Nowak et al. (2016); Zhang and Xu (2017)
Manganese	not reported	4	1.2-1.8	Joshi et al. (2019); Marques-Coelho et al. (2018); Zhang and Xu (2017)
Copper	1.0-9.5	0.77	0.9-1.6	Nowak et al. (2016); Zhang and Xu (2017)
Vitamins (mg/100 g, dry basis)				
vitamin B1 (thiamine)	0.3-0.4	0.01-0.1	0.1-3.3	Marques-Coelho et al. (2018); Rybicka and Gliszczynska-Swiglo (2017); USDA (2005)
Vitamin B2 (riboflavin)	0.3-0.4	0.04-0.41	0.06-10.6	Joshi et al. (2019); Rybicka and Gliszczynska-Swiglo (2017); USDA (2005); Venskutonis and Kraujalis (2013)
Vitamin B3 (niacin)	1.1-1.5	<0.01-8.04	2.1-18.0	Joshi et al. (2019); Marques-Coelho et al. (2018); Rybicka and Gliszczynska-Swiglo (2017); USDA (2005); Zhang and Xu (2017)
Vitamin B6 (pyridoxine)	0.5	0.04-0.6	0.27-0.33	Rybicka and Gliszczynska-Swiglo (2017); USDA (2005); Venskutonis and Kraujalis (2013)
Folate	0.18	0.05-0.07		USDA (2005); Venskutonis and Kraujalis (2013)
Vitamin E (mg/kg, dry basis)	24.7	15.4	9.5-16.4	Alvarez-Jubete et al. (2009); Zhang and Xu (2017)
Total carotenoids (mg/kg, dry basis)	4.6-4.8	3.7-4.7		Tang et al. (2016); Tuan et al. (2013)
Lutein	5.8-12.0	3.6-4.4	3.71	Tang et al. (2016); Tuan et al. (2013)
Zeaxanthin	0.3-5.4	trace-0.3	Not detected	Tang et al. (2016); Tuan et al. (2013)
β-carotene	nd-1.1	nd	1.05	Tang et al. (2016); Tuan et al. (2013)

Table 2. Phenolic diversity of pseudocereal seeds

Pseudocereals	Phenolic class	Phenolic subclass	Phenolic Profile	References
Quinoa	Phenolic acids	Hydroxycinnamic acids	Sinapic acid, Avenanthramide 2f, *p*-Coumaroyl glycolic acid, 2-*S*-Glutathionyl caftaric acid, *p*-Coumaric acid ethyl ester, 24-Methyllathosterol ferulate, *p*-Coumaric acid 4-*O*-glucoside/*p*-Coumaroyl glucose, 24-Methylcholesterol ferulate, *p*-Coumaroyl malic acid, 5-Caffeoylquinic acid, 3/4-Caffeoylquinic acid, 1,2,2'-Triferuloylgentiobiose, 5-5'-Dehydrodiferulic acid, *p*-Coumaric acid, Hydroxycaffeic acid, 24-Methylenecholestanol ferulate, Cinnamoyl glucose, *p*-Coumaroyl tyrosine, Sinapine, Feruloyl glucose, Schottenol ferulate/Sitosterol ferulate, Caffeic acid, Isoferulic acid/Ferulic acid, 3,4/3,5-Diferuloylquinic acid	Gomez-Caravaca et al. (2011); Han et al. (2019b); Rochetti et al. (2017, 2019); Tang et al. (2015)
		Hydroxybenzoic acids	Gallic acid 3-*O*-gallate, 2,4-Dihydroxybenzoic acid, Protocatechuic acid, 2,3-Dihydroxybenzoic acid/Gentisic acid, 3,5-Dihydroxybenzoic acid/2,6-Dihydroxybenzoic acid, 4-Hydroxybenzoic acid 4-*O*-glucoside	
		Hydroxyphenylpropanoic acids	Dihydro-*p*-coumaric acid/Cinnamic acid	
		Hydroxyphenylacetic acids	Methoxyphenylacetic acid, 3,4-Dihydroxyphenylacetic acid/Vanillic acid	
	Flavonoids	Anthocyanins	Pelargonidin, Cyanidin 3-*O*-glucosyl-rutinoside, Petunidin 3-*O*-rutinoside/Pelargonidin 3-*O*-sophoroside/Cyanidin 3-*O*-rutinoside, Cyanidin 3-*O*-sambubioside 5-O-glucoside, Pelargonidin 3,5-*O*-diglucoside, Delphinidin 3-*O*-glucoside, Peonidin, Delphinidin 3-*O*-rutinoside/Cyanidin 3-*O*-sophoroside/Cyanidin 3,5-*O*-diglucoside, Delphinidin 3-*O*-sambubioside, Cyanidin 3-O-sambubioside, Delphinidin 3-*O*-glucosyl-glucoside/Delphinidin 3,5-*O*-diglucoside, Cyanidin 3-*O*-(6"-*p*-coumaroyl-glucoside), Cyanidin 3-*O*-(6"-caffeoyl-glucoside)/Delphinidin 3-*O*-(6"-*p*-coumaroyl-glucoside), Cyanidin, Cyanidin 3-*O*-(6"-malonyl-3"-glucosyl-glucoside), Pelargonidin 3-*O*-(6"-malonyl-glucoside), Pelargonidin 3-*O*-glucosyl-rutinoside, Malvidin 3-*O*-(6"-caffeoyl-glucoside), Petunidin 3-*O*-arabinoside/Peonidin 3-*O*-arabinoside/Cyanidin 3-*O*-glucoside, Pelargonidin 3-*O*-glucoside	
		Dihydrochalcones	Phloretin	
		Dihydroflavonols	Dihydromyricetin 3-*O*-rhamnoside, Dihydroquercetin 3-*O*-rhamnoside/Eriodictyol 7-*O*-glucoside, Dihydroquercetin,	
		Flavanones	Neohesperidin/Hesperidin, Didymin/Poncirin, Naringin 6'-malonate, Hesperetin, Sakuranetin, Naringenin/Butein	
		Flavones	Apigenin 6-C-glucosideCirsimaritin, Nepetin, Luteolin 7-*O*-(2-apiosyl-glucoside), Tetramethylscutellarein, Nobiletin, 7,4'-Dihydroxyflavone, Geraldone, Apigenin 7-*O*-(6"-malonyl-apiosyl-glucoside), 6-Hydroxyluteolin 7-*O*-rhamnoside, Rhoifolin 4'-*O*-glucoside, Chrysoeriol 7-O-apiosyl-glucoside/Luteolin 7-O-rutinoside/Apigenin 6,8-di-C-glucoside, Chrysin, Hispidulin/Apigenin, Luteolin 7-*O*-glucoside, 6-Hydroxyluteolin, Luteolin 6-C-glucoside, Tangeretin, Luteolin/Scutellarein, Apigenin 6,8-C-galactoside-C-arabinoside,	

Tabla 2 (*Continued*)

Pseudocereals	Phenolic class	Phenolic subclass	Phenolic Profile	References
Quinoa	Flavonoids	Isoflavonoids	Genistin, Glycitein/Biochanin A, Daidzein, 6"-*O*-Acetyldaidzin	Gomez-Caravaca et al. (2011); Han et al. (2019b); Rochetti et al. (2017, 2019); Tang et al. (2015); Ross et al. (2017); Smeds et al. (2007)
		Flavanols	(+)-Catechin, (-)-Epicatechin-(2a-7)(4a-8)-epicatechin 3-O-galactoside, (-)-Epicatechin, (+)-Catechin 3-*O*-gallate, Theaflavin, (-)-Epicatechin 3-*O*-gallate, Prodelphinidin dimer B3	
		Flavonols	Spinacetin 3-*O*-glucosyl-(1-6)-glucoside, 3-Methoxysinensetin, Kaempferol, Kaempferol 3-*O*-rhamnosyl-rhamnosyl-glucoside/Kaempferol 3-*O*-xylosyl-rutinoside, Kaempferol 3-*O*-xylosyl-glucoside, Methylgalangin, Kaempferol 3-*O*-galactoside 7-*O*-rhamnoside/Kaempferol 3-*O*-rutinoside, Quercetin 3-*O*-glucuronide, Kaempferol 3-*O*-glucosyl-rhamnosyl-glucoside, Isorhamnetin/Rhamnetin, Quercetin 3-*O*-xylosyl-rutinoside, Quercetin/Morin, Kaempferol 3-*O*-(2"-rhamnosyl-galactoside) 7-*O*-rhamnoside, Quercetin 3-*O*-galactoside/glucoside, Quercetin 3-*O*-rhamnoside/Kaempferol 3-*O*-glucoside/Kaempferol 3-*O*-galactoside, Quercetin 3-*O*-galactoside 7-*O*-rhamnoside/Quercetin 3-*O*-rhamnosyl-galactoside/Kaempferol 3,7-*O*-diglucoside/Kaempferol 3-*O*-sophoroside/Quercetin 3-*O*-rutinoside, Quercetin 4'-*O*-glucoside/Myricetin 3-*O*-rhamnoside, Quercetin 3-*O*-arabinoside, Quercetin 3-*O*-rhamnosyl-rhamnosyl-glucoside, Quercetin 3-*O*-glucosyl-xyloside, Patuletin 3-O-glucosyl-(1-6)-[apiosyl(1-2)]-glucoside	
	Lignans	Furofurans	Sesamolin/Episesaminol/SesaminolSecoisolariciresinol-sesquilignan, Sesamol, 7-Hydroxysecoisolariciresinol, Sesamolinol, Episesamin/Sesamin, syringaresinol, lariciresinol, 7-Hydroxymatairesinol	
		DBL	7-Oxomatairesinol	
	Other polyphenols	Alkylmethoxyphenols	4-Ethylguaiacol	
		Alkylphenols	5-Tricosenylresorcinol, 4-Ethylphenol, 4-Vinylphenol, 5-Heptadecylresorcinol, 5-Pentacosenylresorcinol, 5-Heneicosylresorcinol, 5-Heneicosenylresorcinol, 4-Ethylcatechol, 5-Nonadecylresorcinol, 5-Nonadecenylresorcinol, 3/4-Methylcatechol, 5-Tricosylresorcinol, 1,3-dihydroxy-5-heneicosylbenzene, 1,3-dihydroxy-5-methyleicosylbenzene a, 1,3-dihydroxy-5-methyleicosylbenzene b	
		Tyrosols	Tyrosol, *p*-HPEA-EDA, Hydroxytyrosol 4-*O*-glucoside, Hydroxytyrosol, *p*-HPEA-AC	
		Furanocoumarins	Isopimpinellin, Psoralen	
		Hydroxybenzaldehydes	Gallic aldehyde, p-Anisaldehyde, Syringaldehyde, Protocatechuic aldehyde	
		Hydroxycinnamaldehydes	Ferulaldehyde/Mellein	
		Hydroxyphenylpropenes	2-Methoxy-5-prop-1-enylphenol, Eugenol, Estragole	
		Methoxyphenols	Guaiacol	
		Naphtoquinones	Juglone, 1,4-Naphtoquinone	
		Phenolic terpenes	Epirosmanol/Rosmanol, Carnosic acid, Carvacrol/Thymol	
		Hydroxybenzoketones	2,3-Dihydroxy-1-guaiacylpropanone	
		Hydroxycoumarins	Coumarin	
	Stilbenes	Stilbenes	Piceatannol	

Tabla 2 (*Continued*)

Pseudocereals	**Phenolic class**	**Phenolic subclass**	**Phenolic Profile**	**References**
Amaranth	Phenolic acids	Hydroxybenzoic acids	4-Hydroxybenzoic acid 4-*O*-glucoside, , 2/3/4-Hydroxybenzoic acid	Rocchetti et al. (2017, 2019)
		Hydroxycinnamic acids	Stigmastanol ferulate, Sinapine, Ferulic acid 4-O-glucoside, Feruloyl glucose,Schottenol ferulate/Sitosterol ferulate, caffeic acid, Isoferulic acid/Ferulic acid, 3,4/3,5-Diferuloylquinic acid	
		Hydroxyphenylpropanoic acids	Dihydro-*p*-coumaric acid/Cinnamic acid	
		Hydroxyphenylacetic acids	Methoxyphenylacetic acid, 3,4-Dihydroxyphenylacetic acid/Vanillic acid	
	Flavonoids	Anthocyanins	Malvidin 3,5-*O*-diglucoside, Pelargonidin, Pelargonidin 3-*O*-sambubioside, Petunidin 3-*O*-(6"-p-coumaroyl-glucoside)/Pinotin A, Malvidin 3-*O*-galactoside, Cyanidin 3-*O*-glucosyl-rutinoside, etunidin 3-*O*-rutinoside/Pelargonidin 3-*O*-sophoroside/Cyanidin 3-*O*-rutinoside, Cyanidin 3-*O*-sambubioside 5-*O*-glucoside	
		Dihydrochalcones	3-Hydroxyphloretin 2'-*O*-xylosyl-glucoside	
		Flavanone	Neohesperidin/Hesperidin	
		Flavone	Apigenin 6,8-C-arabinoside-C-glucoside/Apigenin 7-*O*-apiosyl-glucoside, Cirsimaritin, Chrysoeriol 7-*O*-apiosyl-glucoside/Luteolin 7-*O*-rutinoside/Apigenin 6,8-di-C-glucoside, Apigenin 6,8-C-galactoside-C-arabinoside	
		Flavanols	(+)-Catechin, Prodelphinidin trimer C-GC-C/GC-C-C, Prodelphinidin trimer GC-GC-C, (-)-Epicatechin-(2a-7)(4a-8)-epicatechin 3-*O*-galactoside, (-)-Epicatechin	
		Flavonols	Kaempferol 3-*O*-(2"-rhamnosyl-6"-acetyl-galactoside) 7-*O*-rhamnoside, Spinacetin 3-*O*-glucosyl-(1-6)-glucoside, Kaempferol 3-O-galactoside 7-*O*-rhamnoside/Kaempferol 3-*O*-rutinoside	
	Lignans	Furofurans	Secoisolariciresinol-sesquilignan, Sesamol	
		DBL	Anhydro-secoisolariciresinol	
	Other polyphenols	Alkylmethoxyphenols	4-Ethylguaiacol, 5-Tricosenylresorcinol, 5-Pentadecylresorcinol, 4-Ethylphenol, 4-Vinylphenol, 5-Heptadecylresorcinol, 5-Pentacosenylresorcinol, 5-Heneicosylresorcinol, 5-Heneicosenylresorcinol, 4-Ethylcatechol, 5-Nonadecylresorcinol, 5-Nonadecenylresorcinol	
		Tyrosols	Tyrosol, *p*-HPEA-AC	
		Furanocoumarins	Xanthotoxin/Bergapten, Isopimpinellin	
		Phenolic terpenes	Carnosic acid, Carnosol, Rosmadial, Carvacrol/Thymol	
		Hydroxyphenylpropenes	Acetyl eugenol, Anethole, Estragole	
		Hydroxycoumarins	4-Hydroxycoumarin/Umbelliferone, Coumarin	
		Other polyphenols	Phlorin, Pyrogallol	
		Hydroxybenzoketones	2,3-Dihydroxy-1-guaiacylpropanone	
		Hydroxybenzaldehydes	Protocatechuic aldehyde	

Tabla 2 (*Continued*)

Pseudocereals	**Phenolic class**	**Phenolic subclass**	**Phenolic Profile**	**References**
Buckwheat	Phenolic acids	Hydroxycinnamic acids	Sinapic acid, Avenanthramide 2c, Avenanthramide 2f, Avenanthramide 2p, p-Coumaric acid ethyl ester, p-Coumaric acid 4-O-glucoside/p-Coumaroyl glucose, 1-Sinapoyl-2,2'-diferuloylgentiobiose, 3,5-Dicaffeoylquinic acid, p-Coumaroyl malic acid, Rosmarinic acid, 5-Caffeoylquinic acid, 3/4-Caffeoylquinic acid, 4-Hydroxyphenylacetic acid, 3/4/5-Feruloylquinic acid, Caffeic acid ethyl ester, p-Coumaric acid, 3,4/4,5-Dicaffeoylquinic acid, Avenanthramide K, p-Coumaroyl tyrosine, Caffeic acid, Isoferulic acid/Ferulic acid, 3,4/3,5-Diferuloylquinic acid, 3,4-Dihydroxyphenylacetic acid/Vanillic acid, Dihydrocaffeic acid/Homovanillic acid, 3/4/5-Feruloylquinic acid	Liu et al. (2019); Ölschläger et al. (2008); Rochetti et al. (2019); Zielinska et al. (2012)
		Hydroxyphenylacetic acids	Homoveratric acid, Methoxyphenylacetic acid	
		Hydroxybenzoic acids	Benzoic acid, Gallic acid ethyl ester/Syringic acid, 4-Hydroxybenzoic acid 4-O-glucoside	
		Hydroxyphenylpropanoic acids	Dihydro-*p*-coumaric acid/Cinnamic acid	
	Flavonoids	Anthocyanins	Pelargonidin, Cyanidin 3-*O*-glucosyl-rutinoside, Cyanidin 3-*O*-sambubioside 5-*O*-glucoside, Pelargonidin 3,5-*O*-diglucoside, Pelargonidin 3-O-rutinoside, Delphinidin 3-*O*-glucoside, Delphinidin 3-*O*-arabinoside/xyloside, Delphinidin 3-*O*-rutinoside/Cyanidin 3-*O*-sophoroside/Cyanidin 3,5-*O*-diglucoside, Peonidin 3-*O*-(6"-acetyl-galactoside), Delphinidin 3-*O*-glucosyl-glucoside/Delphinidin 3,5-*O*-diglucoside, Delphinidin 3-*O*-(6"-acetyl-glucoside), Delphinidin 3-O-(6"-acetyl-galactoside), Cyanidin 3-*O*-(6"-caffeoyl-glucoside)/Delphinidin 3-*O*-(6"-*p*-coumaroyl-glucoside), Malvidin 3-*O*-glucoside, Cyanidin 3-*O*-(6"-malonyl-glucoside), Pelargonidin 3-*O*-(6"-malonyl-glucoside), Petunidin 3-*O*-(6"-acetyl-glucoside), Pigment A, Peonidin 3-*O*-(6"-acetyl-glucoside), Peonidin 3-*O*-(6"-*p*-coumaroyl-glucoside), Pelargonidin 3-*O*-glucoside	
		Dihydrochalcones	Phloretin, 3-Hydroxyphloretin 2'-*O*-glucoside,	
		Dihydroflavonols	Dihydroquercetin 3-*O*-rhamnoside/Eriodictyol 7-*O*-glucoside	
		Proanthocyanins	epicatechingallate, epicatehin-3-*O*-dimethylgallate, procyanidin B2, procyanidin B5, epiafzelechin-(4–6)-epicatechin, epiafzelechin-(4–8)-epicatechin-*p*-OH-benzoate, epiafzelechin-(4–8)-epicatechin-methylgallate, epiafzelechin-(4–8)-epicatechin-(3,4-dimethyl)-gallate, epicatechin-(4–8)-epicatechin-*O*-(3,4-dimethyl)-gallate, epiafzelechin-(4–8)-epiafzelechin-(4–8)-epicatechin, epiafzelechin-(4–8)-epiafzelechin-(4–8)-epicatechin-*O*-(3,4-dimethyl)-gallate	
		Flavanones	Neohesperidin/Hesperidin, Eriodictyol, Pinocembrin, Naringin 6'-malonate, Hesperetin, Neoeriocitrin/Eriocitrin, Naringenin/Butein	
		Flavones	Cirsimaritin, Nepetin, Cirsilineol, 5,6-Dihydroxy-7,8,3',4'-tetramethoxyflavone, Luteolin 7-*O*-(2-apiosyl-glucoside), Eupatorin, Apigenin 6-C-glucoside, Apigenin 7-*O*-(6"-malonyl-apiosyl-glucoside), Rhoifolin 4'-*O*-glucoside, Pebrellin, 7,3',4'-Trihydroxyflavone, Hispidulin/Apigenin, Isorhoifolin/Rhoifolin, Sinensetin, Jaceosidin, 6-Hydroxyluteolin, Tangeretin	

Tabla 2 (*Continued*)

Pseudocereals	**Phenolic class**	**Phenolic subclass**	**Phenolic Profile**	**References**
Buckwheat	Flavonoids	Isoflavonoids	Genistin, 6"-*O*-Acetylglycitin, 6"-*O*-Acetyldaidzin, Genistein/Baicalein, 6"-*O*-Acetylgenistin	Liu et al. (2019); Ölschläger et al. (2008); Rochetti et al. (2019); Zielinska et al. (2012); Smeds et al. (2007)
		Flavanols	Galangin, (+)-Catechin, (-)-Epicatechin-(2a-7)(4a-8)-epicatechin 3-*O*-galactoside, (-)-Epicatechin, (+)-Catechin 3-*O*-glucose, Theaflavin, Prodelphinidin dimer B3, Spinacetin 3-*O*-glucosyl-(1-6)-glucoside, Kaempferol 3-*O*-rhamnosyl-rhamnosyl-glucoside/Kaempferol 3-*O*-xylosyl-rutinoside, Quercetin 3-*O*-(6"-acetyl-galactoside) 7-*O*-rhamnoside, Kaempferol 3-*O*-xylosyl-glucoside, 3,7-Dimethylquercetin, Isorhamnetin 3-*O*-glucoside 7-*O*-rhamnoside, Quercetin 3-*O*-xylosyl-rutinoside, Isorhamnetin/Rhamnetin, Quercetin/Morin, Kaempferol 3-*O*-(2"-rhamnosyl-galactoside) 7-*O*-rhamnoside, Quercetin 4'-*O*-glucoside/Myricetin 3-*O*-rhamnoside, Jaceidin 4'-*O*-glucuronide	
	Lignans	Furofurans	Secoisolariciresinol-sesquilignan, Sesamol, 1-Acetoxypinoresinol, Lariciresinol-sesquilignan, Sesamolinol, Episesamin/Sesamin, Syringaresinol, lariciresinol, secoisolariciresinol	
		DBL	7-Oxomatairesinol, Conidendrin	
	Other polyphenols	Alkylmethoxyphenols	4-Vinylsyringol, 4-Vinylguaiacol	
		Alkylphenols	5-Tricosenylresorcinol, 4-Ethylphenol, 4-Vinylphenol, 5-Heptadecylresorcinol, 5-Pentacosenylresorcinol, 5-Heneicosylresorcinol, 4-Ethylcatechol, 5-Nonadecylresorcinol, 5-Nonadecenylresorcinol, 3/4-Methylcatechol, 5-Tricosylresorcinol	
		Tyrosols	Tyrosol, 3,4-DHPEA-AC, Oleoside 11-methylester, Oleuropein-aglycone, Oleuropein, Hydroxytyrosol, *p*-HPEA-AC	
		Curcuminoids	Demethoxycurcumin	
		Hydroxybenzaldehydes	*p*-Anisaldehyde, Vanillin, 4-Hydroxybenzaldehyde, Syringaldehyde, Protocatechuic aldehyde	
		Hydroxybenzoketones	3-Methoxyacetophenone, 2,3-Dihydroxy-1-guaiacylpropanone	
		Hydroxycinnamaldehydes	Sinapaldehyde, Ferulaldehyde/Mellein,	
		Hydroxycoumarins	Scopoletin	
		Hydroxyphenylpropenes	2-Methoxy-5-prop-1-enylphenol, Eugenol, Acetyl eugenol, Estragole	
		Methoxyphenols	Guaiacol	
		Naphtoquinones	Juglone	
		Hydroxycoumarins	4-Hydroxycoumarin/Umbelliferone, Coumarin	
		Other polyphenols	Phlorin, Pyrogallol	
		Phenolic terpenes	Carvacrol/Thymol	
	Stilbenes	Stilbenes	Resveratrol, Resveratrol 5-*O*-glucoside, Pterostilbene, Resveratrol 3-*O*-glucoside	

Table 3. Biological activity of pseudocereals protein hydrolyzates and peptides

Pseudocereal	**Source**	**Strategy**	**Identified peptides**	**Biological activity**	**Reference**
Amaranthus cruentus	Protein concentrate	Alcalase hydrolysis + simulated gastrointestinal digestion	---	ACE inhibitory activity	Tiengo et al. (2009)
	Protein isolate	Simulated gastrointestinal digestion	GGV; IVG; VGVL	Hypocholesterolemic activity by inhibiting the activity of HMG-CoA reductase	Manólio Soares et al. (2015)
Amaranthus hypochondriacus	Albumin, globulin, and prolamin	Trypsin hydrolysis + simulated gastrointestinal digestion	PPPP; GP; PP; MP; VA; MA; KA; LA; FA; AP; FP PA, LP, VP, LL, VV, HA, IPA, IPI	DPP-IV inhibitory activity	Velarde-Salcedo et al. (2013)
	Extruded amaranth flour	Simulated gastrointestinal digestion	---	Anti-inflammatory activity in LPS-induced macrophages by preventing activation of NF-κB signaling	Montoya-Rodríguez et al. (2014a)
	Extruded amaranth flour	Simulated gastrointestinal digestion	HGSEPFGPR RPRYPWRYT RDGPFPWPWYSH	Anti-atherosclerotic effect in LPS-induced THP-1 macrophages by reducing the expression of proteins associated with LOX-1 signaling pathway	Montoya-Rodríguez et al. (2014b; 2015)
	Albumin and globulin	Alcalase hydrolysis	---	ACE inhibitory and radical scavenging activity, and iron reducing power DPP-IV inhibitory activity and control of postprandial glycemia in STZ-induced diabetic mice	Soriano-Santos & Escalona-Buendía, (2015) Soriano-Santos et al. (2015)
	Protein isolate	Alcalase hydrolysis	SSEDIKE IADEDPDEANDK	Anti-inflammatory effects in colonic epithelial cells the NF- κ B signaling pathway Inhibition of the allergy reaction in a mouse food allergy model, with suppression of IgE secretion and control of the intestinal inflammation preventing the NF-κB activation	Moronta et al. (2016a) Moronta et al. (2016b)
	Protein isolate	Endogenous protease hydrolysis	---	*In vitro* antithrombotic and antioxidant activity	Sabbione et al. (2016a)

Table 3. (*Continued*)

Pseudocereal	Source	Strategy	Identified peptides	Biological activity	Reference
Amaranthus hypochondriacus	Protein isolate	Simulated gastrointestinal digestion	IQAEAGLTEV TEVWDSNEQE IDTANHANQLD AFEDGFE NDQGQSVFDEELS IDTANHANQLDK LAGKPQQEHSGEHQ DVYTPE DNLNDPK LVDGNDPR AESSQIDTGSK	*In vitro* antithrombotic activity by fibrin clotting inhibition	Sabbione et al. (2016b)
	Protein isolate	Alcalase hydrolysis	QAFEDGFEWVSFK AFEDGFEWVSFK SFNLPILR FNLPILR SFNLPIL VNVDDPSKA	*In vitro* antihypertensive activity by renin competitive inhibition	Quiroga et al. (2017)
A. mantegazzianus	Albumins, globulins, globulins P, and glutelins	Alcalase hydrolysis	---	Antioxidant activity by radical scavenging ability and ability to inhibit linoleic acid oxidation	Tironi and Añón, (2010)
	Protein isolate	Enzymatic hydrolysis	---	ACE inhibitory activity and antihypertensive effects in SHR	Fritz et al. (2011)
	Protein isolate	Alcalase hydrolysis + simulated gastrointestinal digestion	---	*In vitro* antioxidant activity against reactive species	Orsini Delgado et al. (2015)
	Protein isolate	Simulated gastrointestinal digestion	AWEEREQGSR YLAGKPQQEH IYIEQGNGITGM TEVWDSNEQ	Oxygen radical antioxidant capacity	Orsini Delgado et al. (2016)

Table 3. (*Continued*)

Pseudocereal	Source	Strategy	Identified peptides	Biological activity	Reference
A. mantegazzianus	Protein isolate	Simulated gastrointestinal digestion	TEVWDSNEQ IYIEQGNGITGM LAGKPQQEHSGEHQ YLAGKPQQEH LQAEQDDR HVIKPPSRA AWEEREQGSR AVNVDDPSK GDRFQDQHQ KFNRPETT HVIKPPSRA	Prevention of *in vitro* LDL oxidation	García Fillería and Tironi, (2017)
Chenopodium quinoa	Protein isolate	Alcalase hydrolysis	---	ACE inhibition and radical scavenging activity	Aluko and Monu, (2003)
	Protein isolate	Papain and microbial papain-like enzyme hydrolysis	---	DPP-IV inhibitory and radical scavenging activity	Nongonierma et al. (2015)
	Quinoa dough fermented with *Lactobacillus plantarum*	---	IVLVQEG; TLFRPEN; VGFGI; FTLIIN; LENSGDKKY	Radical scavenging activity, inhibition of linoleic acid autoxidation, and protective effects against oxidative stress induced in human keratinocytes	Rizzello et al. (2017)
	Protein concentrate	Simulated gastrointestinal digestion	IQAEGGLT; DKDYPK; GEHGSDGNV; IFQEY;	Anti-diabetic activity by DPP-IV, α-amylase and α-glucosidase inhibition	Vilcacundo et al. (2017)
			SFFVFL; RELGEWGI; GGLGDVLGGLP	Radical scavenging activity, and colon cancer cell inhibitory activity	Vilcacundo et al. (2018)
	Protein isolate	Alcalase hydrolysis	---	Radical scavenging activity, reducing power, and metal ion chelating activity	Li et al. (2018a)
Fagopyrum esculentum Moench	Protein isolate	Alcalase hydrolysis	---	Antioxidant activity by radical scavenging ability, reducing power and ability to inhibit linoleic acid peroxidation	Tang et al. (2009)
	Albumin and glutelin	Simulated gastrointestinal digestion	---	DPP-IV inhibitory activity	Wang et al. (2015)

ACE: angiotensin converting enzyme
LDL: low density lipoproteins
LOX-1: lectin-like oxidized low-density lipoprotein receptor-1
LPS: lipopolysaccharide
SHR: spontaneously hypertensive rats
STZ: streptozotocin

Table 4. Health benefits of pseudocereals demonstrated by animal and human models

Pseudocereal	**Disease**	**Animal model**	**Human model**	**Design of study**	**Observed outcomes**	**References**
Amaranth	Hypertension	Spontaneously hypertensive rats	---	Intragastric administration of protein hydrolyzate 52 animals	* Reduction of blood pressure	Fritz et al. (2011)
	---	Wistar rats	---	Basal *vs* amaranth protein diet in cholesterol-enriched diets 42 animals 4 weeks	* Reduction of plasma total cholesterol and TG * Increase of faecal cholesterol excretion * Antioxidant effects by increasing FRAP values, and decreasing of TBA value in plasma and liver and SOD activity	Lado et al. (2015)
	Diabetes	Streptozotocin-induced CD1 mice	---	Amaranth protein hydrolyzates 25 animals 30 min (acute study) 4 weeks (chronic study)	* Significant improvement of glucose tolerance * Increments in plasma insulin in acute and chronic studies	Soriano-Santos et al. (2015)
	---	Wistar albino rats	---	Basal *vs.* amaranth-based diet 24 animals 15 days	* Significant reduction of food intake and body weight gain * Decrease of plasma ghrelin levels and increase of plasma postprandial leptin and cholecystokinin levels * Improvement in blood glucose profile	Mithila & Khanum, (2015)
	Fe-deficiency	Wistar albino rats	---	Control *vs.* amaranth bread	* Increase of Fe content * Significant increase of haemoglobin concentrations * Down-regulation of transferrin receptor 2 (TfR2) and IL-6 expression levels * Influence on Fe bioavailability	Laparra and Haros, (2016)
	Cardiovascular disease	Wistar rats	---	Casein *vs.* amaranth protein 22 animals 2 weeks	* Significant induction of clotting tests, thrombin time, and activated partial thromboplastin time	Sabbione et al. (2016c)
	Obesity	C57BL/6 mice	---	Basal *vs.* amaranth protein diet in diet-induced obesity 48 animals 8 weeks	* Increase caecal crypt depth and calceiform cells number * Reduction of abundance of *Ruminococcaceae* and *Lachnospiracea* induced by high fat diet * Induction of abundance of *Prevotellaceae*	Olguín-Calderón et al. (2019)

Table 4. (*Continued*)

Pseudocereal	Disease	Animal model	Human model	Design of study	Observed outcomes	References
Buckwheat	High fat diet	C57BL/6 mice	---	Control, and high fat diet with casein *vs* tartary buckwheat protein 27 animals 6 weeks	* Reduction of plasma total cholesterol and TG * Promotion of the growth of *Lactobacillus*, *Bifidobacterium*, and *Enterococcus* * Inhibition of the growth of *E. coli* * Decrease of the levels of plasma inflammation factors (LPS, TNF-α, and IL-6) * Increase of the excretion of total bile acids and short-chain fatty acids in feces	Zhou et al. (2018)
	Type-2 diabetes	---	Diabetic subjects	Buckwheat *vs* rice crackers Acute or chronic (1 week) consumption	* Alteration of post-prandial plasma GLP-1, GIP, and pancreatic polypeptide after buckwheat acute consumption * No changes in post-prandial glucose, insulin or C-peptide concentrations * No effects of consuming buckwheat on fasting glucose, lipids or apolipoproteins in healthy and diabetic subjects	Stringer et al. (2013)
	Type-2 diabetes	---	Diabetic patients	Control *vs.* tartary buckwheat group (150 g/day) 165 individuals 4 weeks	* Decrease of fast insulin, total cholesterol, and LDL-cholesterol * No changes in blood glucose or glycated hemoglobin levels	Qiu et al. (2016)
Quinoa	High-fructose diet	Wistar rats	---	Control *vs.* quinoa seeds in high-fructose diet 24 animals 5 weeks	* Significant reduction of serum total, and LDL-cholesterol, and TG * Reduction of glucose and total protein levels in plasma * Inhibition of HDL-decrease induced by fructose	Pasko et al. (2010)
	Diet-induced obesity	C57BL/6J mice	---	20-hydroxyecdysone-enriched extract 48 animals 3 weeks	* Reduction of adipose tissue through reduction of adipocyte size and decrease in the expression of genes involved in lipid storage * Attenuation of mRNA levels of inflammation (monocyte chemotactic protein-1, CD68) and insulin resistance (osteopontin, PAI-1) markers * Reversion of the effects of high fat-induced down-regulation of mRNA muscle levels of protein UCP(s)	Foucault et al. (2011)

Table 4. (*Continued*)

Pseudocereal	**Disease**	**Animal model**	**Human model**	**Design of study**	**Observed outcomes**	**References**
Quinoa	Diet-induced obesity	C57BL/6J mice	---	20-hydroxyecdysone-enriched extract 48 animals 3 weeks	* Increase of energy expenditure without affecting food intake and activity * Increase of glucose oxidation leading to an increased respiration quotient value * Increase in fecal lipid excretion without any change in stool amount	Foucault et al. (2014)
	Fe-deficiency	Wistar albino rats	---	Control *vs.* quinoa bread	* Increase of Fe content * Significant increase of haemoglobin concentrations * Down-regulation of transferrin receptor 2 (TfR2) expression levels	Laparra and Haros, (2016)
	---	*C. elegans*	---	Phytoecdysteroid-enriched quinoa (1 mg/mL)	* Increase of median lifespan * Improvement of locomotory performance * Enhancement of basal respiration rate * Reduction of advances glycation end-product pigments, ROS, and body fat	Graf et al. (2017)
	Colonic colitis	C57BL/6 mice	---	Control *vs* quinoa diet 40 animals DSS induction (5 days exposure. 2.5%)	* Lessen of clinical symptoms with reduction of disease activity index and the degree of histological damage * Alleviation of the DSS-induced dysbiosis with decrease of abnormal expansion of Proteobacteria * Decrease of overgrowth of *Escherichia/Shigella* and *Peptoclostridum*	Liu et al. (2018)
	Obesity-associated inflammation	Obese diabetic db/db mice	---	Basal diet *vs* quinoa-diet 30 animals 8 weeks	* Reduction of total and LDL cholesterol * Reduction of oxidized LDL * Reduction of protein carbonyls * Decrease of IL-6 * Diminution of hepatic steatosis and total cholesterol accumulation in liver	Noratto et al. (2019)
	High glycemic index diets	Wistar rats	---	Sprouted or fermented quinoa flours High glycemic index diets 36 animals 47 days	* Reduction of food intake, blood glucose, and lipid levels * Accumulation of epididymal adipose tissue	De Oliveira Lopes et al. (2019)

Table 4. (*Continued*)

Pseudocereal	**Disease**	**Animal model**	**Human model**	**Design of study**	**Observed outcomes**	**References**
Quinoa	Malnutrition	---	Undernourished children	Quinoa infant food (200 g/day) 40 individuals 15 days	* Increase of IGF-1 in plasma	Ruales et al. (2002)
	Cardiovascular disease	---	Students	Quinoa bars (19.5 g/day) 22 individuals 30 days	* Reduction of total and LDL-cholesterol and TG * No significant reduction of blood glucose levels, body weight and blood pressure	Farinazzi-Machado et al. (2012)
	Postmenopause	---	Postmenopausal and overweight women	Quinoa *vs.* corn flakes 35 individuals 4 weeks	* Significant reduction in serum TG, TBARS, and vitamin E concentrations * Increase in urinary excretion of enterolignans * Reduction of total, and LDL-cholesterol * Increase in GSH levels	De Carvalho et al. (2014)
	Obesity	---	Overweight and obese subjects	Quinoa seeds (25 and 50 g/day) 50 individuals 12 weeks	* No alteration of body composition, nutrient intake, and total, LDL, and HDL cholesterol * Decrease of TG in quinoa group * Reduction of prevalence of metabolic syndrome	Navarro-Pérez et al. (2017)
	Prediabetes	---	Prediabetic subjects	Control *vs.* processed quinoa 30 individuals 28 days	* Significant decrease of body mass index and glycated hemoglobin * Increase in the satiation and fullness (complete) degree * No significant differences in fasting plasma glucose levels	Abellan Ruiz et al. (2017)
	Prediabetes	---	Prediabetic subjects	Combination of quinoa and foxtail millet 10 individuals 75 days	* Reduction of total, HDL, and VLDL-cholesterol and TG * No modification of HDL cholesterol	Anusha et al. (2018)
	Cardiovascular disease	---	Overweight male subjects	Quinoa enriched bread *vs.* refined wheat bread 37 individuals 8 weeks	* Modification of glucose response * Minimal effects on other cardiovascular risk biomarkers (LDL-cholesterol)	Li et al. (2018b)

Table 5. Recent gluten-free (GF) products developed (2009 onwards)

GF foods	Ingredients	Remarks	References
Bread	Base of rice flour and xantan gum with 50% of quinoa, amaranth or buckwheat flours	Replacement of potato starch by pseudocereal flours resulted in softer bread crumbs an higher protein, minerals, vitamin E and phenolics contents and antioxidant activity than control GF bread (50% potato starch instead of pseudoceal flours) Quinoa and buckwheat flours enhanced bread volume	Alvarez-Jubete et al. (2009, 2010a,b)
	Base of corn starch replaced by 10%, 20%, 30% and 40% of buckwheat flour	Buckwheat flour increased magnesium, phosphorous, potassium and phenolic compounds content and the antioxidant activity compared to GF control bread. Breads containing 40% of buckwheat flour showed the highest overall sensory quality	Wronkowska et al. (2010)
	Base of rice flour replaced by 10%, 20%, 30% of husked or unhusked buckwheat flour	Husked buckwheat flour decreased starch retrodegradation, enhanced anti-staling properties and provides a pleasant flavor and taste	Torbica et al. (2010)
	60-70% popped amaranth flour and 30-40% raw amaranth flour	High-quality bread loves are produced in terms of volume and crumb structure	Calderón de la Barca et al. (2010)
	Base of rice flour replaced by 10%, 20%, 30% of light or whole grain buckwheat flours	Whole grain buckwheat flour increased the phenolics content and antioxidant activity compared to light buckwheat flour	Sakač et al. (2011)
	Commercial GF formulation and HPMC replaced by DBF (40%) or combination of DBF (35%) + PBF (5%)	Inclusion of 40% of DBF improved the leavening properties and the baking performance of a commercial GF bread mixture. Addition of limited amounts of PBF and HPMC improved the crumb structure of GF bread formulations.	Mariotti et al. (2013)
	100% buckwheat or 100% quinoa flour breads	Buckwheat breads exhibited lower glycemic index than wheat-based bread	Wolter et al. (2013)
	Base of rice and corn flour replaced by 40-100% of quinoa white flour	Quinoa white flour enhanced the specific volume and produced homogeneous crumb structure of bread without adversely affects the taste	Elgeti et al. (2014)

Table 5. (*Continued*)

GF foods	Ingredients	Remarks	References
Bread	Base of rice flour, potato starch, cassava starch and sour tapioca starch replaced by 20% of quinoa or amaranth whole flours	Quinoa and amaranth breads presented similar specific volume, firmness and water activity to control bread but showed higher protein, lipid and ash content and larger alveolar area.	Machado Alencar et al. (2015)
	100% buckwheat flour bread	Buckwheat bread enhances contents of iron, zinc, calcium potassium, phosphorus, magnesium and cupper compared with wheat bread Buckwheat flour decreased bread specific volume and increased bread hardness compared to wheat bread Sensory properties of 100% buckwheat bread were acceptable	Sayed et al. (2016)
Flatbread	Mixture of quinoa, peanut oilcake and broccoli/beet	Higher protein and mineral contents and acceptance than wheat-based breads	Khalon et al. (2019)
Cookies	20% of popped amaranth flour and 13% of whole grain popped amaranth	Cookies with high protein content and expansion factor and hardness comparable to other GF cookies	Calderón de la Barca et al. (2010)
	Base of rice flour replaced by 10%, 20%, and 30% of buckwheat flour	Addition to 10-20% of buckwheat flour produced cookies with the highest sensory scores and cohesive structure	Torbica et al. (2012)
	Mixture of quinoa flour, quinoa flakes and corn starch	Mixture containing 30% quinoa flour, 25% quinoa flakes and 45% corn produced cookies rich in dietary fiber, essential amino acids, linolenic acid and minerals and with good sensory acceptability	Brito et al. (2015)
	100% of raw or germinated amaranth flour	Cookies obtained with raw and germinated amaranth flour showed higher spread ratio than wheat-based cookies Germinated amaranth produced cookies with the highest antioxidant activity and total dietary fiber, minimum snap force and acceptable sensory attributes.	Chauhan et al. (2015)
	100% buckwheat flour with 1% of gums	Buckwheat biscuits showed higher moisture content, thickness, and weight and decreased fracture strength than wheat-based biscuits. Biscuits from buckwheat flour and xanthan gum exhibited good sensory attributes	Kaur et al. (2015)

Table 5 (*Continued*)

GF foods	**Ingredients**	**Remarks**	**References**
Cookies	100% quinoa flour	Quinoa cookies produced with optimized variables (fat content, sugar content, baking temperature and time) exhibited good textural and sensory quality and high antioxidant activity	Jan et al. (2018)
	Base of rice flour replaced by 30% of tartary buckwheat malt or flour	Addition of buckwheat flour brought about cookies with higher total phenolic, rutin and quercetin contents and antioxidant activity and lower glycemic index	Molinari et al. (2018)
Muffins	Base of rice flour replaced by 25%, 50%, 75% and 100% of quinoa flour	Inclusion of quinoa flour up to 75% produced good quality muffins regarding texture and overall acceptability	Bhaduri (2013)
Bakery products	Base of rice and oat flour replaced by 5%, 10% and 15% of roasted quinoa flour	Addition of upto10% quinoa flour provided GF bakery products with good sensory attributes	Kaur & Kaur, (2017)
Crackers	Mixture of refined or whole grain buckwheat flour and corn meal (70:30 w/w)	Crackers made from buckwheat flours have higher tocopherol and phenolic contents and antioxidant activity than wheat-based crackers without adversely affects their sensory quality	Sedej et al. (2011)
Wafer sheet	Base of rice replaced by 20%, 40% or 60% corn, chestnut or buckwheat flours	Wafer sheet formulations containing rice-buckwheat flours (60:40) had the closest value of consistency, flow behavior index, hardness and fracturability to wheat-based wafer sheet.	Mert et al. (2015)
Snacks	Whole grain quinoa flour with spices	Quinoa-based snacks exhibited good water activity, textural and sensory acceptability.	Khalon et al. (2016)
Vermicelli-type pasta	pre-gelatinized flour made from cassava starch and cassava bagasse (70:30), native cassava starch and amaranth flour (10:60:30)	The pasta developed showed acceptable color, texture and nutritional value comparable to that of commercial wheat products. The pasta showed better cooking quality and lower solids loss than commercial GF pasta.	Fiorda et al. (2013a,b)
Fresh spaghetti	Mixture of dried potato pulp, extruded potato pulp and amaranth flour at different concentrations	Mixture containing dried potato pulp, extruded potato pulp and amaranth flour (65:10:25) provided fresh pasta with higher yield, better color and cooking characteristics than fresh commercial wheat pasta.	Bastos et al. (2016)
Pasta	Base of rice flour replaced by quinoa and amaranth flours at different concentration and egg white	Formulation containing amaranth, quinoa and rice flours (10:40:50) exhibited good elasticity and sensory acceptability but poor toughness	Makdoud & Rosentrater, (2017)

Table 5 (*Continued*)

GF foods	**Ingredients**	**Remarks**	**References**
Noodles	Mixture of potato starch, extruded and non-extruded quinoa (red and white flour) an tara gum with or without addition of lupine flour, vegetable proteins and POx-enzyme	Formulation containing quinoa flour and lupine flour (70:30) in combination with rice protein (12%) and POx-enzyme (1%) showed satisfying noodle quality and high protein and fiber contents	Linares-García et al. (2019)
Beer-like beverage	100% buckwheat malt	The GF beer developed showed pH, FAN, fermentability and total alcohol comparable to wheat beers and acceptable sensory quality	Nic Phiarais et al. (2010)
	100% buckwheat or quinoa malt	Buckwheat beers showed similar fermentability values, the wort pH and soluble protein content similar to barley beer Quinoa beer showed similar viscosity and beverage pH to barley beer and highest levels of metal cations Beers containing pseudocereals showed good general acceptance	Deželak et al. (2014)
Symbiotic fermented beverage	Quinoa and soy water-extracts at different proportions fermented with 2% of *L. casei* LC-1	Formulations containing 70-100% of quinoa showed higher viscosity and consistency Formulation containing 30% of quinoa showed the least hysteresis, the best sensory acceptance and chemical composition and the highest intention to purchase	Bianchi et al. (2014)
Fermented beverage	Buffalo skim milk replaced by 25-100% of quinoa water-extract	Addition of 75-100% of quinoa produced fermented beverages with good sensory quality and high content of several amino acids and minerals.	El-Deeb et al. (2014)
	Quinoa seeds from Rosada de Huanaayo and Pasankalla varieties fermented with differente lactic acid bacteria strains	Fermented quinoa-based beverages showed high protein, fiber, vitamins and minerals levels and viable and stable microbial counts during storage microbial load during storage time.	Ludena-Urquizo et al. (2017)

*DBF: dehulled bucksheat flour; FAN: free amino nitrogen; HPMC: hydroxypropylmethylcellulose; PBF: puffed buckwheat flour; POx: Pyranose 2-oxidase

Highlights

* Quinoa, amaranth, and buckwheat are the most representative pseudocereals.

* High nutritional value has made pseudocereals become a modern trend in human diet.

* Multiple health benefits have been attributed to pseudocereal grains.

* Pseudocereals are gluten-free grains suitable for development of foods for celiacs.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.