REVIEW

Pseudocereals dietary fiber. Amaranth, quinoa, and buckwheat fiber composition and potential prebiotic effect

Fibra dietaria en pseudocereales. Composición y potencial efecto prebiótico de la fibra de amaranto, quinoa y trigo sarraceno

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Abstract

Amaranth (*Amaranthus*), buckwheat (*Fagopyrum esculentum*), and quinoa (*Chenopodium quinoa*) crops have limited production and agro-industrial development both in Argentina and globally. As the demand for functional ingredients and foods grows, developing products from these pseudocereals could offer substantial economic benefits. This study aims to analyze the dietary fiber content and composition of amaranth, quinoa, and buckwheat, and to investigate the relationship between dietary fiber structure and its potential prebiotic effects. Gaining insights into these aspects would provide valuable information for developing foods based on these pseudocereals and could enhance their future applications in the food industry.

Keywords: Pseudocereals • dietary fiber • prebiotic effect • microbiota

Resumen

Los cultivos de amaranto (*Amaranthus*), el trigo sarraceno (*Fagopyrum esculentum*) y la quinoa (*Chenopodium quinoa*) tienen una producción y desarrollo agroindustrial limitados tanto en Argentina como a nivel mundial. Dado que la demanda de ingredientes y alimentos funcionales está en aumento, el desarrollo de productos a partir de estos pseudocereales podría ofrecer beneficios económicos sustanciales. Este estudio tiene como objetivo analizar el contenido y la composición de la fibra dietética del amaranto, la quinoa y el trigo sarraceno, y examinar la relación entre la estructura de la fibra dietética y sus posibles efectos prebióticos. Obtener información sobre estos aspectos proporcionaría datos valiosos para el desarrollo de alimentos basados en estos pseudocereales y podría potenciar sus aplicaciones futuras en la industria alimentaria.

Palabras clave: Pseudocereales • fibra dietaria • efecto prebiótico • microbiota

Pseudocereals

The pursuit of healthy lifestyles and more nutritious foods adaptable to various climatic conditions has spurred interest in underutilized or alternative crops, leading to a resurgence in pseudocereals. The World Health Organization (WHO), the Food and Agriculture Organization of the United Nations (FAO), and the scientific community are working together to identify foods that can meet the needs of a growing global population. With a current population of 8 billion and projections reaching 10.9 billion by 2050, urgent solutions are needed to address the impending food crisis (7). The agroindustry faces the challenge of ensuring a sufficient food supply while upholding high productivity and quality standards.

According to the FAO, "food security exists when all people, at all times, have physical, social, and economic access to sufficient, safe, and nutritious food that meets their daily energy needs and dietary preferences for an active and healthy life." Currently, global food security relies on a few cereal varieties, with over 50% of caloric intake provided by wheat, maize, and rice (50). Pseudocereals are nutritionally superior to traditional cereals. They have higher protein content and are rich in essential amino acids, including lysine, arginine, tryptophan, and histidine. Furthermore, pseudocereals exhibit higher digestibility, bioavailability, and protein efficiency ratios (PER), comparable to milk casein (50). Unlike wheat, oats, barley, and rye, which contain gliadin, pseudocereals are gluten-free and safe for celiac patients (43).

Ongoing research in food science uncovers new, healthy food components. Bioactive peptides, found in various foods including pseudocereals, exemplify this discovery. These peptides, along with other beneficial components, classify pseudocereals as functional foods (45). Lipids, another crucial nutritional component, exhibit high unsaturation levels in pseudocereals (75-86%). Linoleic acid (omega-6) is the predominant fatty acid, followed by oleic and palmitic acids, with notable amounts of linolenic acid (omega-3) (54). Both linoleic and linolenic acids are essential for the body, offering benefits such as cardiovascular disease prevention and improved insulin sensitivity (43). Pseudocereals also provide substantial dietary fiber, akin to whole grains.

This fiber supports gastrointestinal health, aids in weight management, and reduces the risk of non-communicable diseases like diabetes and cardiovascular conditions (2). Additionally, pseudocereals are richer in minerals such as magnesium, calcium, zinc, iron, copper, and phosphorus compared to cereals (table 1) (50).

Table 1. Centesimal composition and mineral content of amaranth, quinoa, buckwheat pseudocereals, and wheat.

Tabla 1. Composición centesimal y contenido de minerales de amaranto, quinoa, trigo sarraceno y trigo.

Despite the potential benefits that pseudocereals offer, several factors still hinder the incorporation of these crops into global agri-food systems. These factors are diverse and include social aspects, economic factors like low market participation and lack of integration into mass consumer products, as well as agronomic factors like yield and lack of technology applied to these crops. Knowledge regarding pseudocereals yield and quality is restricted to small-scale systems with low investment cultivated in rustic ways, and therefore cannot be compared to mass crops knowledge. The abundance of technology and research available for traditional cereals, combined with numerous and established marketing channels, leads producers to choose not to invest in underutilized crops.

Pseudocereals centesimal composition

Table 1 presents the proximate composition ranges for wheat and pseudocereals such as amaranth, quinoa, and buckwheat for comparison. Their compositional values and technological and culinary behaviors are similar (4). Starch, which forms semi-crystalline granules, is the primary component in both pseudocereals and wheat. Although cereals generally have lower protein content, pseudocereals lack gluten-forming proteins, making their flour unsuitable for traditional baked goods.

Lipid content is typically higher in amaranth and quinoa compared to cereals. These pseudocereals have stable lipids due to high concentrations of tocopherols (4). As shown in table 1, dietary fiber content is similar between pseudocereals and wheat. Fiber levels largely depend on whether the seed is hulled, as most fiber is located in the outer coverings. Both cereals and pseudocereals can be consumed as whole grains, whole grain flour, or processed products. While whole seeds are becoming more popular, most wheat and rice consumed by humans are dehulled, resulting in lower fiber content.

Table 1 displays the mineral content in wheat and pseudocereals. Pseudocereals generally contain higher levels of calcium, magnesium, and iron. According to the Argentine Food Code (CAA) Recommended Daily Intake (RDI) values, a 50 g serving of wheat provides only 2% of the RDI for calcium, while the same serving of amaranth provides 9%. For magnesium, a 50 g serving of wheat provides 19% of the RDI for men (260 mg/day), whereas 50 g of pseudocereals provide 40% to 54% of the RDI. Amaranth is particularly notable for its iron content, offering 16% of the RDI for women (29 mg/day) and 33% of the RDI for men (14 mg/day) in a 50 g serving. These values demonstrate that pseudocereals are a valuable source of minerals.

*Data taken from Haros and Schoenlechner (2017), and Serna-Saldívar and Sanchez-Hernandez (2020b). Data expressed as g/100 g of dry weight.

*Data taken from Alvarez-Jubete *et al.* (2010). Data expressed as mg/100 g of dry weight. *Valores tomados de Haros y Schoenlechner (2017) y Serna-Saldívar y Sanchez-Hernandez (2020b). Datos expresados como g/100 g en peso seco.

*Datos tomados de Alvarez-Jubete *et al.* (2010). Datos expresados como mg/100 g en peso seco.

Dietary Fiber

Dietary fiber intake is increasingly recognized for its importance in human nutrition. Research shows that fiber supports proper intestinal function and helps prevent cardiovascular diseases, obesity, diabetes, and certain cancers (2).

The Food and Drug Administration (FDA) recommends adults consume 25 g of fiber daily in a 2000 kcal diet, which aligns with the National Cancer Institute (NCI) recommendation of 20-30 g/day to prevent colon cancer (36). However, statistics from Argentina show that the population fails to meet these recommendations. The 2007 National Nutrition and Health Survey reported that 97.2% of women aged 10 to 49 did not meet the daily fiber intake recommendation, with a median intake of 9.4 g/day. Similar deficiencies were observed in children, with 97.8% not meeting adequate fiber intake (42). Data from the 2019 National Nutrition and Health Survey suggest that the situation has not improved. Food consumption patterns reveal that 30-40% of respondents consume only one fruit or vegetable per day, indicating that dietary fiber intake remains significantly below recommended levels (42).

There are several definitions of dietary fiber. According to the CAA, "dietary fiber is any edible material not hydrolyzed by the endogenous enzymes of the human digestive tract." Compaore-Sereme *et al.* (2022) define it as "the edible parts of plants or carbohydrates resistant to digestion and absorption in the human small intestine, with complete or partial fermentation in the large intestine," highlighting its fermentability by large intestine microorganisms. Dietary fiber consists mainly of non-starch polysaccharides in plant cell walls and can be classified based on water-holding capacity into insoluble and soluble fiber. Insoluble fiber includes cellulose and hemicelluloses, while soluble fiber encompasses pectins, β-glucans, gums, mucilages, oligosaccharides, and inulin. Additionally, dietary fiber includes indigestible non-polysaccharide compounds such as lignin, proteins resistant to gastrointestinal digestion, phenolic compounds, waxes, saponins, phytates, and phytosterols (52). Health benefits are linked to the solubility of dietary fiber. Soluble dietary fiber (SDF) forms viscous gels upon contact with water, which delays gastric emptying and nutrient absorption in the intestines. This increases satiety and reduces caloric density, lowering the long-term risk of obesity (72). The delay in nutrient absorption also helps prevent glycemic spikes in diabetic patients. Additionally, SDF improves insulin sensitivity in both type 2 diabetes patients and healthy individuals (69), and lowers blood cholesterol levels, particularly LDL cholesterol, thereby reducing cardiovascular disease risk (45). This effect may result from SDF binding bile acids, altering micelle formation, preventing bile acid reabsorption in the enterohepatic circulation, and promoting their elimination in feces (74). Consequently, new bile acid synthesis in the liver is stimulated, which lowers blood cholesterol levels (8). Another important aspect of fiber is its fermentability by intestinal microorganisms. The degree of fermentability correlates with fiber solubility and particle size. For example, fructooligosaccharides are highly fermentable, whereas large cellulose or lignin polymers remain unchanged throughout the large intestine. Clinical studies show that intestinal microorganisms utilize different types of dietary fiber, with the remainder excreted in feces (18). Soluble polysaccharides fermented by intestinal microbiota produce short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate. SCFAs offer various health benefits, primarily at the intestinal level. Butyrate, for instance, strengthens the intestinal epithelial barrier by inducing tight junction protein expression and redistribution within the membrane. A loss of intestinal barrier integrity, leading to increased permeability, is linked to chronic inflammation associated with obesity, insulin resistance, and type 2 diabetes (9). Propionate also benefits individuals with obesity by inhibiting hepatic cholesterol synthesis, decreasing lipogenesis in adipose tissue, and reducing appetite (9). Insoluble dietary fiber (IDF) travels through the gastrointestinal tract with minimal modification. Its effects are largely due to mechanical interactions (69). IDF retains water and adds bulk to feces, enhancing intestinal regularity. Additionally, it reduces caloric density by acting as a physical barrier that slows the transit of digestive products through the enterocytes' brush border (69). This characteristic of insoluble dietary fiber (IDF) also impedes the absorption of other components, such as cholesterol, and promotes its excretion via feces. Studies have shown that the lignin fraction, commonly found in the outer layers of seeds, enhances bile acid binding capacity, leading to reduced blood cholesterol levels (46).

Sabbione *et al.* (2023a) reported that amaranth IDF has significant bile acid binding capacity, suggesting that its adsorptive effect may contribute to a potential hypocholesterolemic effect by sequestering bile acids. Additionally, IDF reduces the concentration and contact time of potentially carcinogenic compounds with the colon mucosa, thereby lowering the risk of colon cancer (5). Regarding other cancers, research indicates that total or insoluble dietary fiber from legumes reduces prostate cancer risk, while soluble dietary fiber decreases breast cancer risk (64).

Pseudocereals dietary fiber composition

Numerous studies have assessed the relative amounts of total dietary fiber (TDF), soluble dietary fiber (SDF), and insoluble dietary fiber (IDF) in pseudocereal whole grains and flours (table 2). These values vary widely due to differences in genotypes, environmental conditions, and laboratory techniques used for fiber quantification and characterization. While dietary fiber content is crucial, the ratio between insoluble and soluble fractions is also significant, as an appropriate balance enhances health benefits. The FDA recommends an IDF/SDF ratio close to 3 for optimal fiber balance (45). Understanding the polysaccharide structures in pseudocereal cell walls is important, as these structures influence both techno-functional and biological properties. This information can predict the behavior of fiber in the body and its effects on various food matrices.

Table 2. Amaranth, quinoa, and buckwheat dietary fiber content, expressed as total dietary fiber (TDF), soluble dietary fiber (SDF), and insoluble dietary fiber (IDF). **Tabla 2.** Contenido de fibra dietaria total (TDF), soluble (SDF) e insoluble (IDF) en amaranto, quinoa, y trigo sarraceno.

Data are expressed as g fiber/100 g of seeds on a wet basis. *IFDaverage/ SFD^{average}

Valores expresados como g fibra/100 g de semillas. *IFDmedia/ SFDmedia

> Over the past decades, various studies have focused on extracting and quantifying dietary fiber (DF) from fruits and vegetables to identify rich sources, assess their health benefits, and evaluate their functional properties for product development. Notable achievements have been observed in citrus fruits, tropical fruits, berries, and various vegetables.

Cell wall constituents

Dietary fiber components are primarily located in the cell wall, which provides shape and structural integrity to plant cells. The cell wall consists of a complex mixture of polysaccharides and other polymers arranged in a three-dimensional network. It also includes structural proteins, enzymes, phenolic polymers, and other materials that influence its physical and chemical properties. The characteristics of the cell wall, such as thickness, matrix arrangement, and the types and proportions of molecules, vary depending on the plant tissue (41).

Among the cell wall constituents is *cellulose*, a linear polysaccharide made up of D-glucose molecules linked by β-D (1-4) bonds. Its degree of polymerization varies significantly, with molecules consisting of 2,000 to 15,000 units, depending on the specific region of the cell wall (66). These linear polymers form microfibrils through intermolecular hydrogen bonds, which aggregate into larger structures and interact with other components such as hemicelluloses, pectins, and lignin. This interaction creates strongly hydrated matrices with high mechanical resistance. *Hemicelluloses* are a diverse group of polysaccharides with long linear chains that may have short side chains as substituents. They have a lower molecular weight than cellulose and are generally more soluble. However, their physicochemical properties, such as solubility, viscosity, and gel-forming abilities, vary based on their chemical structure, molecular size, interactions, and spatial arrangement. At least 250 types of hemicellulose polymers have been identified, including xyloglucans, xylans, mannans, glucans, and β-(1-3,1-4) glucans (53). *Lignins*, another cell wall component, are complex biopolymers and are the second most abundant in the plant kingdom after cellulose. These polyphenolic biopolymers consist of phenylpropane units and form a matrix through the condensation of three primary phenolic alcohols (47). *Pectins* are structural polysaccharides primarily composed of α-D-galacturonic acid. They form chains that can be linear, known as homogalacturonans (HG), or include rhamnose. Two typical structures containing rhamnose are rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II), which differ in structure, linkage types, and complexity. Although pectins vary in solubility, the pectin in the middle lamella of cell walls is insoluble and considered calcium pectate (75). These compounds have significant nutritional and technological value for the food industry due to their prebiotic potential and gelling ability (66). *Fructans*, which are reserve carbohydrates and the most abundant non-structural polysaccharides in nature, include inulin, oligofructose, and fructooligosaccharides (FOS). They are relatively soluble in water and remain intact in the upper gastrointestinal tract. However, in the colon, fructans are fully utilized by microorganisms, providing a prebiotic effect. Inulin, FOS, and oligofructose are commonly used as functional ingredients (70). These compounds also offer valuable technological properties, such as sweetening ability and gel formation, which enhance the body and palatability of certain foods. *Oligosaccharides*, which are short chains of sugars containing 3 to 20 monosaccharides, cannot be digested by the human body and are classified as dietary fiber. Due to their small size and capacity to form hydrogen bonds with water, they are highly soluble. The *raffinose family of oligosaccharides* (RFOs), including raffinose, stachyose, and verbascose, as well as related compounds, have shown potential prebiotic effects by promoting the selective growth of beneficial bifidobacteria (35). *Resistant starch* (RS) is a type of starch that retains its structural characteristics and remains undigested as it passes through the gastrointestinal tract. It reaches the colon, where it can be fermented by the microbiota or excreted in feces. Although RS is not a cell wall component, it functions similarly to fermentable soluble fiber, providing associated health benefits (34).

Amaranth

Table 2 (page 167) presents the values of total dietary fiber (TDF), insoluble dietary fiber (IDF), and soluble dietary fiber (SDF) for amaranth, quinoa, and buckwheat, expressed on a wet basis. The TDF values align with those reported for pseudocereals in table 1 (page 165). The data show a predominance of IDF over SDF. Specifically, 12% to 42% of the TDF corresponds to soluble fiber, while 58% to 86% corresponds to insoluble fiber.

Bunzel *et al.* (2005) reported that the primary cell walls of dicotyledons like amaranth are rich in pectins, xyloglucans, and cellulose. Lamothe *et al.* (2015) examined the composition of the insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) fractions in amaranth. They found that 5% of the IDF on a dry basis was lignin. Their analysis revealed that the IDF primarily consists of galacturonic acid, arabinose, xylose, glucose, and galactose. A significant portion of the glucose was attributed to cellulose and xyloglucans, which retained the characteristic bonds of these structures. Glucose from cellulose accounted for 7% of the IDF, though this value might be underestimated due to the analytical method used. The estimated proportion of xyloglucans was 30%, with a high degree of branching indicated by the elevated Xyl/Glc ratio. This finding is consistent with Bunzel *et al.* (2005), who noted a high number of terminal xylose units in amaranth, likely part of the xyloglucan side chains.

Sabbione *et al.* (2023a) confirmed that galacturonic acid is the primary monosaccharide in insoluble dietary fiber (IDF) and reported the same monosaccharides identified by Lamothe *et al.* (2015) (60). Lamothe *et al.* (2015) found that pectins constitute 59% of the IDF, with rhamnose present in low amounts. This suggests that the pectins are predominantly homogalacturonans (HG) with small amounts of rhamnogalacturonan I (RG-I). The bonding patterns in galactose and arabinose residues suggest they are part of RG-I pectic side chains. The predominance of HG over RG-I is consistent with Mohnen (2008), who noted that HG makes up to 65% of the pectin in plant cell walls, while RG-I represents 25-35%. Additionally, Lamothe *et al.* (2015) indicated that xylose is involved not only in xyloglucan structures but also in arabinoxylans, another type of hemicellulose found in the IDF, together with pectic polysaccharides that would be the majority. Regarding amaranth soluble dietary fiber (SDF), Lamothe *et al.* (2015) reported that the primary monosaccharides are galacturonic acid, galactose, and arabinose, indicating the presence of pectic substances. These pectic substances contribute 34% to the SDF (38). The predominance of galacturonic acid and the absence of rhamnose suggest that homogalacturonans (HG) are the major pectin component. Significant amounts of mannose were also reported, though it is notably lower compared to other monosaccharides. This mannose is attributed to galactomannans, with an estimated content of 0.3%. Additionally, xylose and glucose units from xyloglucans also contribute to the SDF, comprising 60-70% of this fiber fraction. Lamothe *et al.* (2015) describe a high Xyl/Glc ratio in these samples, reflecting a high level of branching. The side chains of these polysaccharides include di- and tri-saccharides, which may consist of xylose, glucose, and possibly arabinose. Sabbione *et al.* (2023a) detected galacturonic acid, xylose, arabinose, mannose, and glucose/galactose in amaranth SDF, with xylose and arabinose being the most prevalent. Villacrés *et al.* (2013) also reported significant amounts of soluble arabinoxylans in the SDF.

Capriles *et al.* (2008) studied resistant starch content in amaranth seeds and found that raw seeds possess 0.5% RS on a dry basis. However, they observed that after cooking, this value reduced to 0.2%. The authors concluded that the RS decrease might have occurred due to the starch granules' small size and their tendency to completely lose the crystalline structure during thermal treatment. Since a food considered a good source of resistant starch should possess an RS/total starch ratio of at least 4.5% (58), amaranth cannot be included in that group of foods since its ratio is 0.86%.

Guzmán-Maldonado and Paredes-López (1998) reported low levels of raffinose and stachyose in amaranth, with concentrations of 1.65% and 0.15%, respectively. Gamel *et al.* (2006) found even lower levels of raffinose, around 0.4%, and similar stachyose content. Both studies confirm that amaranth seeds are not a significant source of raffinose family oligosaccharides. Various authors have also assessed the presence of FODMAPs, which include fermentable oligosaccharides, disaccharides, monosaccharides, and polyols like lactose, fructose, sorbitol, and mannitol. These non-digestible carbohydrates can trigger symptoms in individuals with conditions such as irritable bowel syndrome, causing abdominal distension, diarrhea, and pain. The impact of these carbohydrates is limited to those with specific intolerances or diseases. Békés *et al.* (2017) classified pseudocereals, including amaranth, buckwheat, and quinoa, as low in FODMAPs, indicating that their FOS content is also low. Furthermore, Habus *et al.* (2022) analyzed the FODMAPs content in amaranth bran and reported that the sum of fructans and galacto-oligosaccharides (GOS) is 0.96% on a dry weight basis.

Quinoa

Table 2 (page 167) presents the total dietary fiber (TDF), insoluble dietary fiber (IDF), and soluble dietary fiber (SDF) content in quinoa seeds. Across different studies, IDF consistently exceeds SDF, with soluble fiber ranging from 10% to 37% of TDF, and insoluble fiber ranging from 63% to 90% of TDF. Zhang *et al.* (2020) analyzed the dietary fiber content of quinoa seeds, reporting that it contains 41% hemicellulose, 52% cellulose, 4.7% pectins, and 1.7% lignin (77). Lamothe *et al.* (2015) studied the soluble and insoluble fiber composition in quinoa seeds, finding that the IDF contained 9% lignin. This finding aligns with the data from Repo-Carrasco-Valencia and Serna (2011), who reported lignin values between 6% and 7%.

Based on the types of bonds present in the IDF xylose and glucose units, these monosaccharides are likely part of xyloglucans, as suggested by Serna Saldívar and Ayala Soto (2020). These authors identified xyloglucans as the primary hemicellulose components in quinoa seeds. The xyloglucans found in quinoa are similar to those in amaranth, characterized by branching with di- and trisaccharide side chains and a significant degree of branching. In their analysis of IDF, Lamothe *et al.* (2015) reported that glucose associated with cellulose constituted 6%, a value comparable to that found in amaranth. However, this finding is much lower than the 52% TDF described by Zhang *et al.* (2020), suggesting that the lower cellulose content could be attributable to the analytical methods employed (38).

The primary monomeric unit identified in quinoa TDF by Lamothe *et al.* (2015) is galacturonic acid with $β$ -(1,4) linkages, indicating that pectic polysaccharides are predominant in both IDF and SDF fractions. According to these authors, these pectic polymers constitute approximately 55% of IDF, predominantly as homogalacturonans (HG) and, to a lesser extent, as rhamnogalacturonan I (RG-I) compounds, which are branched with arabinans and galactans. Cordeiro *et al.* (2012) also described similar pectic structures in quinoa dietary fiber, reinforcing these findings. Furthermore, the pectic content in SDF constitutes 55%, but unlike the pectins found in IDF, the SDF pectins are composed exclusively of branched homogalacturonans (HG), as rhamnose was absent from this fraction. Lamothe *et al.* (2015) identified arabinose, glucose, galactose, and, to a lesser extent, xylose and mannose in SDF. Most of the arabinose is part of the pectic branches, specifically as arabinans. Galactomannans in quinoa SDF account for 0.5%, while xyloglucans range from 40-60%, exhibiting a low Xyl/Glc ratio and a relatively low degree of branching. Additionally, Villacrés *et al.* (2013) reported significant amounts of soluble arabinoxylans as part of quinoa SDF.

The resistant starch (RS) content in quinoa seeds, as reported by Kraic (2006), is 12.6 g/kg on a dry basis, translating to an RS/total starch ratio of approximately 2%. Consequently, quinoa is not considered a significant source of resistant starch. However, a study by Linsberger-Martin *et al.* (2012) demonstrated that applying high hydrostatic pressures to quinoa seeds could increase the RS content by up to 18 times, presenting a promising method for developing functional ingredients in the food industry. In their analysis of the FODMAP profile of various grains, Ispiryan *et al.* (2020) found that quinoa has a low content of fructans and oligofructans (OFR). Fructans were below the detection limit of their method, and the RFOs were present in minimal amounts (0.09% on a dry basis).

Buckwheat

Table 2 (page 167) presents the total dietary fiber (TDF), soluble dietary fiber (SDF), and insoluble dietary fiber (IDF) contents of both husked and dehusked buckwheat seeds, as well as those of the husk alone. The values for TDF, SDF, and IDF vary among studies, partly due to the inclusion or exclusion of the husk in the analyzed samples. The buckwheat husk is particularly rich in insoluble fiber, and flours made from buckwheat that include the husk typically have high levels of insoluble fiber (19). According to Zhang *et al.* (2020), the fiber composition of buckwheat seeds includes 39.2% hemicellulose, 38.8% cellulose, 20.2% lignin, and 1.8% pectin. Based on the data presented in table 2 (page 167), it is inferred that the buckwheat seeds analyzed likely contained the husk, given the high content of cellulose and lignin, which are characteristic of the husk's insoluble polysaccharides. Wefers *et al.* (2015) analyzed the IDF monosaccharides in dehusked buckwheat and identified significant amounts of galacturonic acid, arabinose, galactose, and to a lesser extent, rhamnose. Many of the monosaccharides found in buckwheat IDF, such as galacturonic acid, arabinose, and galactose, are attributed to the presence of pectic arabinans and galactans, which are linked to rhamnogalacturonan-I (RG-I) segments. The observed galacturonic acid/rhamnose ratio indicates that homogalacturonan (HG) also contributes to buckwheat IDF pectin, though to a lesser extent compared to quinoa and amaranth. This study also suggests a relatively low content of cellulose in buckwheat. The presence of terminal xylose and glucose with β -(1-4) linkages implies that xyloglucans may be present, although xylose could also be part of xylans, albeit in low proportions. Regarding SDF, Izydorczyk and Head (2010) noted that it primarily consists of pectic polysaccharides, xyloglucans, and arabinogalactans. The SDF monosaccharide composition analyzed by Wefers *et al.* (2015) supports the presence of RG-I and indicates higher amounts of HG compared to the IDF.

The authors also identified arabinans in the SDF fraction, which are part of the pectin branches. Additionally, glucose and xylose monosaccharides with linkages consistent with xyloglucan structures were found. A high amount of mannose could be attributed to mannan content. Dziedzic *et al.* (2012) further reported that buckwheat husks are predominantly composed of fiber, with high proportions of insoluble fiber, particularly lignin and cellulose.

Regarding buckwheat's resistant starch content, Kraic (2006) reported an RS content of 38 g/kg on a dry basis and an RS/total starch ratio of 6.5%. Thus, buckwheat can be considered a good source of resistant starch compared to other pseudocereals. The high RS values are likely due to buckwheat's elevated amylose content (27).

Ispiryan *et al.* (2020) found that buckwheat is low in FODMAPs, indicating that it contains a low proportion of both fructans and raffinose family oligosaccharides (RFOs).

Table 3 summarizes the composition of IDF and SDF in amaranth, quinoa, and buckwheat seeds.

Table 3. Polysaccharides in the soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) fractions in amaranth, quinoa, and buckwheat.

Tabla 3. Polisacáridos presentes en la fibra dietaria soluble (SDF) y en la fibra dietaria insoluble (IDF) de amaranto, quinoa y trigo sarraceno.

The data on dietary fiber content in pseudocereals show considerable variability among studies. However, some general trends can be identified. Pseudocereals have TDF levels comparable to those found in wheat (table 1, page 165). The predominant fiber fraction is IDF, reflected in an IDF/SDF ratio greater than 1 (table 2, page 167). An ideal balance, suggested to be around 3, is approached by amaranth, quinoa, and cereals, with some husked buckwheat samples exhibiting even higher ratios. Regarding fiber composition, amaranth and quinoa share similarities in the proportion and types of dietary fiber structures (table 2 page 167 and table 3). The main fiber components in these pseudocereals are pectic polysaccharides, with a lesser amount of xyloglucans. These polysaccharides vary in complexity and branching, contributing to the distinct characteristics of the dietary fiber in each pseudocereal. In contrast, husked buckwheat is notable for its high cellulose and lignin content. The removal of the husk reduces the IDF content, which is reflected in the lower IDF values compared to buckwheat with the husk (table 2, page 167). Overall, while the general composition of dietary fiber in pseudocereals follows certain patterns, the specific characteristics and proportions can vary significantly depending on the pseudocereal and its processing.

*Data from Bunzel *et al.* (2005); Cordeiro *et al.* (2012); Dziedzic *et al.* (2012); Izydorczyk and Head (2010); Lamothe *et al.* (2015); Repo-Carrasco-Valencia and Serna (2011); Sabbione *et al.* (2023a); Serna Saldívar and Ayala Soto (2020a); Villacrés *et al.* (2013); Wefers *et al.* (2015); Zhang *et al.* (2020).

Probiotics and prebiotics

The human gastrointestinal tract hosts a vast number of microorganisms that interact symbiotically with the host. These microorganisms perform essential functions, such as utilizing non-digestible components to produce health-beneficial compounds, maintaining the epithelial barrier, regulating host metabolism, preventing pathogen colonization, and modulating the immune and nervous systems (65). Certain microorganisms, known as probiotics, are used as food supplements to improve health. Probiotics must survive gastrointestinal transit, establish in the intestine, and proliferate. The International Scientific Association for Probiotics and Prebiotics (ISAPP) defines probiotics as "live strains of strictly selected microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (30). Foods containing probiotics are classified as functional foods. Common probiotic microorganisms include *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Bifidobacterium spp*. However, other probiotic species also exist, such as *Lactococcus spp.*, *Streptococcus spp.*, and certain strains of *Saccharomyces* yeast (65). Probiotics and many intestinal microbes utilize dietary fiber. Different types of fiber selectively promote the growth of beneficial microbial colonies and are known as prebiotics. The ISAPP defines prebiotics as "substrates that are selectively utilized by host microorganisms and confer a health benefit" (21). This broad definition encompasses non-digestible dietary carbohydrates and other types of substrates. Prebiotics and probiotics enhance host health by modulating intestinal flora. Research aimed at developing healthier foods has led to the emergence of new functional foods, including synbiotics. Synbiotics are defined as "a mixture of probiotics and prebiotics intended to increase the survival of health-promoting bacteria and to modify intestinal flora and its metabolism" (48).

Human intestinal microbiota

The term "human microbiota" refers to the community of microorganisms residing in the body. Over 70% of the human microbiota is found in the digestive tract, which displays significant variability in microbial diversity and quantity across different regions. The intestinal microbiota contains approximately 100 trillion microorganisms from at least 1,000 different species, and it is estimated to weigh about 200 grams in adults. Only one-third of these microorganisms are common to most individuals, while the remaining two-thirds are unique to each person (17).

According to data from the Human Microbiome Project (National Institutes of Health, USA), the phyla *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Proteobacteria*, which together account for over 90% of the total microbiota (table 4), predominantly inhabit the intestine. The remaining microbiota includes *Fusobacteria*, *Verrucomicrobia*, archaea, yeasts, phages, and protists. The human colon also contains low quantities of pathogens such as *Campylobacter jejuni*, *Salmonella enterica*, *Vibrio cholerae*, and certain strains of *Escherichia coli*.

Table 4. Main bacterial phyla and genera present in the human intestine (17). **Tabla 4.** Principales *phylum* y géneros de bacterias presentes en el intestino humano (17).

Digestive tract colonization begins at birth. The quantity and type of microorganisms evolve until age 3, influenced by environmental factors and dietary patterns. After this period, the intestinal microbiota resembles that of adults in composition, diversity, and functionality. It can be altered during adolescence due to hormonal changes but remains relatively stable in adulthood. After age 65, the microbiota composition shifts, with increased abundance of the *Bacteroidetes phylum* and *Clostridium* from the *Ruminococcaceae* family, in contrast to younger individuals, where *Clostridium* from the *Lachnospiraceae* family is more common. In older adults, the microbiota becomes less diverse and more dynamic, characterized by a higher *Bacteroides*/*Firmicutes* ratio, an increase in *Proteobacteria*, and a decrease in *Bifidobacterium* (68). The intestinal microbiota performs numerous functions and influences various biological processes, both locally and distantly through its metabolites. The bioactive products and metabolites produced by microorganisms during fermentation, known as postbiotics, include short-chain fatty acids (SCFAs), enzymes, vitamins, bioactive peptides, and components of microorganisms or their remnants (63). The microbiota contributes to maintaining mucosal barrier integrity, providing essential nutrients like vitamins, and protecting against pathogens (17). Additionally, the interaction between the microbiota and the immune system in the colonic mucosa is essential for proper immune function. Microbial molecular pattern recognition receptors or microbiota-derived metabolites activate barrier functions and mediator synthesis, regulating intestinal immune cell responses to tolerate beneficial microorganisms and prevent pathogen overgrowth (3). The interaction between the human microbiota and the gut-brain axis is a bidirectional and dynamic communication pathway between the intestine and the brain, mediated through nervous, endocrine, and immune signaling mechanisms (62). The enteric nervous system (ENS) in the intestine comprises over 100 million neurons responsible for basic digestive functions, including motility and mucosal secretion. It communicates with the central nervous system (CNS) primarily via the vagus nerve. The intestinal microbiota stimulates vagus nerve afferent pathways, promotes cytokine release, and modulates the production of neurotransmitters, hormones, and metabolites such as SCFAs (23). Microbiota influences the hypothalamic-pituitary-adrenal axis, regulating cortisol release. Research indicates that high levels of *Lactobacillus rhamnosus* are associated with lower corticosterone levels and improved stress and depression management. Conversely, stress can alter the microbiota profile (23). Dysbiosis may disrupt molecules essential for proper CNS function, potentially linking microbiota imbalances to neurological diseases such as Alzheimer's, Parkinson's, multiple sclerosis, autism spectrum disorders, depression, and anxiety (1).

Colon microorganisms primarily ferment dietary carbohydrates that resist digestion in the gastrointestinal tract. Species such as *Bacteroides*, *Roseburia*, *Bifidobacterium*, and *Enterobacter* produce SCFAs, which serve as an energy source for enterocytes or enter the bloodstream to affect distant organs. Many intestinal anaerobes produce acetate, while *Bacteroidetes* predominantly produce propionate and *Firmicutes* produce butyrate (68). Butyrate is recognized for its anti-inflammatory and anticancer properties; it promotes the proliferation of colonocytes in the crypts and enhances apoptosis and exfoliation in the areas closer to the lumen. Additionally, butyrate supports barrier function regulation and reduces bacterial translocation by contributing to tight junction assembly and mucin synthesis (68). SCFAs also impact hepatic lipid and glucose homeostasis. Propionate, besides serving as an energy source, regulates blood glucose levels by modulating gluconeogenesis in the liver. It enhances insulin sensitivity and reduces cholesterol synthesis rates (29). Acetate, used as an energy source in the intestine, can be transported to peripheral organs or the liver, where it serves as a precursor for cholesterol and long-chain fatty acids. Additionally, SCFAs increase intestinal hormone levels that promote satiety and enhance insulin action on glucose uptake in muscle and adipose tissue. They inhibit *de novo* lipogenesis and lipolysis, reducing plasma-free fatty acids and aiding in body weight control (29).

Microbiota Modulation: Probiotics and Prebiotics

Microbiota modulation involves deliberately altering the composition and activity of intestinal microorganisms to improve health. This can be achieved through dietary changes, probiotics, prebiotics, synbiotics, or, occasionally, antibiotics, either alone or in combination.

Such interventions can enhance the diversity and abundance of beneficial bacteria, restore balance, or reduce pathogenic microorganisms.

Probiotics are incorporated into the diet through fermented foods or dietary supplements. Commonly used probiotic genera include *Lactobacillus* and *Bifidobacterium*. Strains such as *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium bifidum*, and *Bifidobacterium lactis* are naturally present in the intestine. These strains, along with those from *Saccharomyces*, can reduce pathogen adherence to the mucosa (39). Additionally, the metabolic activity of beneficial microorganisms increases SCFA concentrations and decreases colonic pH, which inhibits the growth of pathogenic bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Salmonella enteritidis* (59).

Prebiotics can naturally occur in foods or be added to enhance functional properties. Various dietary fibers serve as energy sources for intestinal microorganisms, but only some selectively promote the growth of beneficial microbiota and probiotics. Prebiotics exert health benefits by stimulating beneficial microorganisms' growth, modulating immune function, and protecting against pathogens. They primarily increase populations of *Bifidobacterium* and *Lactobacillus*, but also stimulate less well-studied beneficial bacteria such as *Akkermansia*, *Eubacterium*, *Propionibacterium*, *Roseburia*, and *Faecalibacterium* (59). The rate and extent of fiber fermentation by microorganisms are crucial for their prebiotic potential. These factors are influenced by fiber solubility, chain size, total surface area, and the structure of the cell wall or food matrix containing the fiber (59). Fructans such as inulin, oligofructose, and FOS, as well as galactans (GOS), are well-recognized prebiotics due to their low degree of polymerization and high solubility. Additionally, oligosaccharides derived from hemicelluloses, such as mannanoligosaccharides (MOS), arabinoxylanoligosaccharides (AXOS), and xylooligosaccharides (XOS), have been proposed as prebiotics because they enhance the growth of beneficial microorganisms and promote SCFAs production (33). Conversely, RFOs have shown prebiotic effects by increasing *Bifidobacteria* and *Lactobacillus* populations while reducing the adhesion and colonization of enteric pathogens (35). The degree of polymerization of polysaccharides and their interactions with other polysaccharides in food matrices can affect their metabolism. Some non-starch polysaccharides, such as pectins and certain hemicelluloses, are considered potentially prebiotic (59). Microorganisms must possess enzymes to degrade glycosidic bonds in polysaccharides into smaller molecules for utilization as carbon sources. For example, xyloglucans are degraded by *Clostridium* in the colon due to its microbiota-specific enzymes, converting them into fermentable oligosaccharides that positively impact the colon microbiota (24). β-glucans enhance the growth of *Bifidobacterium* and *Lactobacillus* strains and modulate SCFA production by increasing *Clostridium histolyticum* and *Prevotella* (65). Arabinoxylans in cereals and pseudocereals can generate AXOS and XOS through enzymatic hydrolysis with xylanases and arabinofuranosidases. Although hydrolysis yields various structures depending on the plant source, arabinoxylans are considered potential prebiotics. Their consumption has been linked to positive immunomodulation and selective growth of probiotic microorganisms such as *Lactobacillus cellobiosus*, *Lactobacillus paracasei*, and *Bifidobacterium spp*. (65). Pectins, a complex family of fermentable polysaccharides, can also promote the growth of *Lactobacillus* and *Bifidobacterium* in the intestine. The effectiveness of pectin utilization by microorganisms depends largely on colon pH and the degree of methoxylation, with low methoxyl pectins fermenting more rapidly (49). *In vivo* studies have shown an increase in *Clostridium* species capable of producing acetate and butyrate in the presence of pectin (65). Additionally, research in rats fed with pectins revealed increased SCFAs and the presence of pectic oligosaccharides as intermediates. These oligosaccharides result from the action of bacterial enzymes such as pectate lyase, polygalacturonase, and pectin esterase (49). Resistant starches are also potentially prebiotic, with promising results from both *in vitro* and *in vivo* studies. Several studies indicate that dietary RS increases the number of beneficial microorganisms, particularly *Bifidobacterium*, and elevates SCFA concentration in the colon (13). This proliferation is attributed to the fermentation of resistant starch degradation products. *Ruminococcus bromii* is a key species for initiating RS degradation, enabling other bacteria to utilize its fermentation products (65).

Potential modulatory and prebiotic effect of pseudocereals' dietary fiber

Amaranth

The prebiotic potential of amaranth remains under investigation. Although literature is limited, results are promising and have generated increased interest in these seeds. Gullón *et al.* (2014) assessed the *in vitro* prebiotic potential of amaranth by quantifying SCFAs, monitoring pH changes, and evaluating microbial population dynamics using adult women's fecal inoculums for fermentation. Amaranth seeds were cooked in water and subjected to simulated gastrointestinal digestion. The study revealed significant modifications in bacterial composition, with notable growth of *Bifidobacterium* spp., *Lactobacillus*, and *Enterococcus*, which are beneficial, as well as *Bacteroides* and *Prevotella*, which produce propionate. An increase in *Clostridium coccoides* and *Eubacterium rectale*, both butyrate producers, was also observed. SCFA production showed a progressive increase over time, with higher concentrations of acetate, followed by propionate and butyrate, indicating high fermentability of the medium where amaranth carbohydrates serve as a carbon source. pH decreased over time, correlating with the production of lactate and formate SCFAs (24). Sabbione *et al.* (2023b) investigated the ability of dietary fibers from three amaranth products to modulate children's fecal microbiota using an *in vitro* fermentation model. They observed changes in fecal microbiota and SCFAs after 24, 48, and 72h of fermentation. Sequencing results at 24h revealed a significant decrease in *Fusobacterium* and enterobacteria compared with the basal medium, accompanied by a notable increase in *Bacteroides* and *Parabacteroides*. These findings confirm that amaranth fibers are fermented by children's fecal microbiota, leading to changes indicative of a potentially prebiotic effect.

Currently, no studies confirm which specific amaranth fiber carbohydrates can beneficially modulate the microbiota or increase SCFA production. However, several components show potential prebiotic effects. Given the high content of pectin and arabinoxylans in amaranth, these compounds could be considered potential prebiotics. Additionally, xyloglucans are abundant in both soluble and insoluble fractions of amaranth dietary fiber, suggesting various hemicellulose sizes and conformations (10, 38). The observed prebiotic effects may be linked to xyloglucans, which can be hydrolyzed by microbiota enzymes into smaller carbohydrates used as a carbon source by beneficial microorganisms.

Quinoa

Quinoa, extensively studied as a pseudocereal, has a well-documented prebiotic effect on its dietary fiber. Gullón *et al.* (2014) reported results similar to those observed with amaranth. Authors described an increase in beneficial bacterial groups, although Faecalibacterium prausnitzii grew less in quinoa compared to amaranth. SCFA levels increased significantly over time, with a higher concentration in quinoa compared to amaranth. The study concluded that quinoa provides a highly fermentable medium conducive to the growth of SCFA-producing beneficial bacteria. In agreement, Zeyneb *et al.* (2021) found a marked increase in SCFAs and a decrease in pH during *in vitro* fermentation of cooked and raw quinoa following simulated gastrointestinal digestion. The authors found that propionate and butyrate were the SCFAs present in the highest concentrations after 24h of fermentation, while acetate was present in lower amounts. This low concentration of acetate, which is typically the most abundant SCFA, may suggest its degradation by other bacteria (76). Additionally, the study reported a positive shift in microbial diversity post-fermentation, with increased levels of beneficial species such as *Bifidobacterium* and *Collinsella*, indicating a potential prebiotic effect. Zeyneb *et al.* (2021) also examined polysaccharides extracted from quinoa and found a greater prebiotic effect compared to digested samples, particularly enhancing the growth of *Bifidobacterium*.

Several carbohydrates in quinoa may contribute to potential prebiotic effects. Cao *et al.* (2020) isolated a quinoa fiber carbohydrate composed of glucose and arabinose units, which exhibited a modulatory effect on the microbiota of rats fed a high-fat diet. A decrease in the *Firmicutes*/*Bacteroidetes* ratio (F/B) was noted, which is favorable as a high F/B ratio is linked to metabolic diseases. Additionally, levels of *Clostridium* and *Proteobacteria*, associated with inflammation and metabolic disorders, also decreased. The authors attributed these effects to the reduction in hyperlipidemia induced by the high-fat diet (42).

Other polysaccharides in quinoa, such as pectin or xyloglucans, may also be fermented by colon microorganisms following enzymatic hydrolysis in the intestine. Multiple carbohydrates can contribute to the observed modulatory effects, with SCFAs produced from fermentation playing a role in this process.

Buckwheat

The prebiotic potential of buckwheat has been investigated in various studies. Préstamo *et al.* (2003) examined the effects of incorporating buckwheat into the diet of rats. Their findings revealed a significant increase in Lactobacillus and *Bifidobacteria*, while potentially pathogenic strains, such as *Clostridium* and enterobacteria, decreased, suggesting a prebiotic effect of buckwheat. In a more recent study, Ren *et al.* (2021) showed that buckwheat supplementation in rats on a high-fat diet positively modulates the microbiota, reducing the *Firmicutes*/*Bacteroidetes* ratio and increasing microbial diversity, which helps reverse dysbiosis. Zhou *et al.* (2019) explored the impact of buckwheat RS on the microbiota by supplementing a high-fat diet with this component. The authors reported increased levels of *Lactobacillus*, *Bifidobacteria*, and *Enterococcus*, alongside inhibition of *Escherichia coli*. Additionally, supplementation with buckwheat RS led to increased SCFA production and significantly lower plasma levels of cholesterol, triglycerides, and glucose. The study concluded that buckwheat RS supplementation inhibited inflammation and prevented insulin resistance and hypertriglyceridemia. Given these findings, RS, a prominent component in buckwheat, may play a key role in its prebiotic effects. RS can be degraded by bacterial amylases into simpler carbohydrates that are fermented by intestinal microorganisms. This fermentation can directly influence the microbiota or produce by-products that benefit other microorganisms, leading to positive modulation. Additionally, other polysaccharides in buckwheat, such as pectins, arabinogalactans, and xyloglucans, may also contribute to these effects.

Conclusions

An exhaustive review was conducted on the structure of dietary fiber in amaranth, quinoa, and buckwheat, focusing on the relationship between their components and potential prebiotic effects. The current literature is limited regarding the modulatory effects of dietary fiber from these pseudocereals on human microbiota. Nonetheless, both *in vitro* and *in vivo* studies have evaluated the prebiotic potential of pseudocereal dietary fiber, showing promising results. These studies consistently demonstrate significant increases in beneficial microbial species, reductions in potentially pathogenic species, and enhanced SCFA production. Overall, the findings underscore the potential prebiotic effects of dietary fiber from amaranth, quinoa, and buckwheat. However, Argentine legislation, specifically Article 1390 of the Argentine Food Code (CAA, Chapter XVII), mandates the identification of functional components with potential prebiotic effects. Promoting and incorporating pseudocereals into processed food products would not only enhance the nutritional value of consumers' diets but also diversify raw material sources. Their consumption could offer health benefits, boost the regional economy of pseudocereal-producing areas, support food sovereignty, and provide consumers with options that align with their health needs and personal values.

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