Proteins of Amaranth (*Amaranthus* spp.), Buckwheat (*Fagopyrum* spp.), and Quinoa (*Chenopodium* spp.): A Food Science and Technology Perspective

Frederik Janssen, Anneleen Pauly, Ine Rombouts, Koen J.A. Jansens, Lomme J. Deleu, and Jan A. Delcour

**Abstract:** There is currently much interest in the use of pseudocereals for developing nutritious food products. Amaranth, buckwheat, and quinoa are the 3 major pseudocereals in terms of world production. They contain high levels of starch, proteins, dietary fiber, minerals, vitamins, and other bioactives. Their proteins have well-balanced amino acid compositions, are more sustainable than those from animal sources, and can be consumed by patients suffering from celiac disease. While pseudocereal proteins mainly consist of albumins and globulins, the predominant cereal proteins are prolamins and glutelins. We here discuss the structural properties, denaturation and aggregation behaviors, and solubility, as well as the foaming, emulsifying, and gelling properties of amaranth, buckwheat, and quinoa proteins. In addition, the technological impact of incorporating amaranth, buckwheat, and quinoa in bread, pasta, noodles, and cookies and strategies to affect the functionality of pseudocereal flour proteins are discussed. Literature concerning pseudocereal proteins is often inconsistent and contradictory, particularly in the methods used to obtain globulins and glutelins. Also, most studies on protein denaturation and techno-functional properties have focused on isolates obtained by alkaline extraction and subsequent isoelectric precipitation at acidic pH, even if the outcome of such studies is not necessarily relevant for understanding the role of the native proteins in food processing. Finally, even though establishing in-depth structure–function relationships seems challenging, it would undoubtedly be of major help in the design of tailor-made pseudocereal foods.

**Keywords:** gluten-free, Osborne classification, protein denaturation and aggregation, pseudocereals, techno-functional properties

**Introduction**

The growth of the world population (United Nations Dept. of Economic and Social Affairs 2015), the increasing concerns over sustainability issues as well as the changes in dietary patterns, which are driven by health or disease outcomes (El Nehir and Simsek 2012) necessitate the development of efficient food systems from vegetable (underutilized) sources (El Nehir and Simsek 2012; Raikos and others 2014; Alemayehu and others 2015). Animal proteins such as those in eggs and milk have high nutritional value and important techno-functional properties, but they are less sustainable than vegetable proteins (Pimentel and Pimentel 2003; Hoffman and Falvo 2004). While pseudocereals are dicotyledonous, true cereals such as wheat are monocotyledonous. The seeds of both pseudocereals and cereals themselves are rich in starch. Amaranth (*Amaranthus* spp.), buckwheat (*Fagopyrum* spp.), and quinoa (*Chenopodium* spp.) are globally the best-known pseudocereals. 2 buckwheat species are cultivated worldwide: common buckwheat (*Fagopyrum esculentum* Moench) and tartary buckwheat (*Fagopyrum tataricum*). Both buckwheat types are closely related to one another but have small differences in growth conditions and leaf and flower anatomy (Cai and others 2016). Figure 1 shows the phylogeny of some true cereals, pseudocereals, edible flowering plants, and legumes. Amaranth and quinoa share a more recent common ancestor and are thus more closely related to one another than to buckwheat. Both
Proteins of amaranth, buckwheat, and quinoa belong to the Amaranthaceae, while buckwheat is a member of the Polygonaceae (Bremer and others 2009). In 2014, the world-wide productions of buckwheat and quinoa were estimated at approximately 2 million and 200000 metric tons, respectively (FAOSTAT 2014). Information on the global production of amaranth is not available in the database of the Food and Agriculture Organization of the United Nations (FAO), but in 2004 was estimated to approximate between 600000 and 1.7 million metric tons (Corke and others 2016). In contrast, the world production of common cereals such as maize, rice, and wheat in 2014 was estimated at 1 billion, 740 million, and 728 million metric tons, respectively (FAOSTAT 2014).

Proteins from amaranth (Venskutonis and Kraujalis 2013; Mota and others 2016), buckwheat (Steadman and others 2001; Bonafaccia and others 2003; Wei and others 2003; Mota and others 2016), and quinoa (Vega-Gálvez and others 2010; Mota and others 2016) have better balanced amino acid compositions and thus higher biological values than those of most cereals. Furthermore, they can safely be consumed by people suffering from celiac disease (Bai and others 2013; Colgrave and others 2015) and are more sustainable than those from animal sources (Pimentel and Pimentel 2003; Alemayehu and others 2015). Amaranth, buckwheat, and quinoa not only have unique amino acid compositions, they are also rich in other constituents important for human health, such as dietary fiber (Steadman and others 2001; Bonafaccia and others 2003; Wei and others 2003; Vega-Gálvez and others 2010; Venskutonis and Kraujalis 2013), antioxidants (Paško and others 2009; Zhu 2016), minerals (Wei and others 2003; Vega-Gálvez and others 2010; Venskutonis and Kraujalis 2013), and vitamins (Bonafaccia and others 2003; Vega-Gálvez and others 2010; Venskutonis and Kraujalis 2013).

Because of the above-mentioned aspects, it is not a surprise that numerous studies have been published on the general structure, denaturation, and aggregation behavior of pseudocereal proteins, and also on their techno-functional properties (such as solubility, foaming, emulsifying, and gelling). We here provide the 1st comprehensive food science and technology perspective of the current knowledge on these subjects for amaranth, buckwheat, and quinoa. In addition, an overview is given of the technological impact of pseudocereals on quality attributes of bread, pasta, noodles, and cookies. Finally, crucial knowledge gaps and/or misconceptions are highlighted and perspectives for further research that could benefit the general use of pseudocereals in the food industry are suggested. It is expected that a better understanding of the process-induced structural changes of pseudocereal proteins, their aggregation, and their techno-functional properties will provide a solid scientific basis for the design of high-quality food products based on pseudocereals.

Osborne-Type Fractionation and Structural Properties

Osborne (1907) developed a fractionation scheme which has stood the test of time. Indeed, it is still frequently used to classify cereal grain proteins into different fractions based on their sequential extractability or lack thereof in different media. Albumins are extractable in water, globulins in aqueous salt solutions (such as 0.4 M NaCl), and prolamins in aqueous alcohol solutions (such as 60% (v/v) ethanol). The remaining insoluble fraction contains the glutelins, which are partly extractable in dilute acids or bases (Belitz and others 2009). The relative distribution of amaranth, buckwheat, and quinoa proteins in Osborne-type fractionation schemes reveals that they are more closely related to legume than to cereal proteins (Table 1) (Segura-Nieto and others 1999).

In wheat and many other cereals, prolamins and glutelins are the most abundant protein fractions (Table 1). Especially in wheat, they play a prominent techno-functional role (Delcour and others 2012). In contrast, most legume proteins such as those from soy and pea are extractable in water or salt solutions and thus belong to the albumin or globulin fractions. Literature on protein distributions in Osborne fractions of pseudocereals is inconsistent (Table 1).
In addition, some proteins in amaranth are classified as albumins, even if they are not extracted from flour with water but enriched in the residue after the 3rd extraction (as with aqueous alcohol) as glutelin (Belitz and others 2009), some authors only classify the part of these proteins that are extractable in dilute acids or bases as glutelin. Table 2 lists some characteristics of albumins, globulins, and prolamins.
Table 2—Structural properties reported for Osborne protein fractions from amaranth, common and tartary buckwheat, and quinoa.

<table>
<thead>
<tr>
<th>Albumins</th>
<th>A</th>
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<tbody>
<tr>
<td><strong>General structure</strong></td>
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<tr>
<td>Proteins of amaranth, buckwheat, and quinoa...</td>
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<tr>
<td>Common buckwheat:</td>
<td>Mostly 2S albumins with MM between 8 and 12 kDa. A minority of the 2S albumins has a MM of 16 kDa. The proteins were not linked by an SS-bond.</td>
<td>One major 2S albumin with MM around 12 kDa, with 2 SS-linked subunits of 3 and 8 kDa.</td>
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<td>Tartary buckwheat:</td>
<td>Proteins with MM of 57 and 64 kDa which consist of SS-linked subunits with MM between 14 and 22 kDa, and proteins with MM around 38 and 41 kDa.</td>
<td>In addition, various proteins within the range of 25 to 83 kDa have been detected.</td>
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<tr>
<td><strong>pI</strong></td>
<td>7.5</td>
<td>?</td>
<td>?</td>
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<tr>
<td><strong>Secondary structure</strong></td>
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<tr>
<td>Common buckwheat:</td>
<td>4% α-helices 2% α-helices 37% β-sheets 55% aperiodic structure</td>
<td>2% α-helices 46% β-sheets 52% aperiodic structure</td>
<td>4% α-helices 50% β-sheets 46% aperiodic structure</td>
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<td><strong>Globulins</strong></td>
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<td><strong>General structure</strong></td>
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<tr>
<td>Major 11S globulin</td>
<td>“Amarantin” with MM of 300 kDa and consisting of 6 similar subunits (52 to 59 kDa), each consisting of an acidic (34 to 36 kDa) and a basic (22 to 24 kDa) protein linked by an SS-bond.</td>
<td>Major 13S globulin</td>
<td>“Chenopodin” with MM of 320 kDa and consisting of 6 similar subunits (52 to 59 kDa), which each consist of an acidic (30 to 40 kDa) and a basic (20 to 25 kDa) protein linked by an SS-bond.</td>
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<tr>
<td>Minor 2S globulin</td>
<td>Vicilin-like proteins with apparent MM of 186.0 kDa made up of 8 noncovalently linked subunits: 90.1, 70.9, 40.0, 37.4, 35.2, 31.2, 23.6, and 15.6 kDa.</td>
<td>Minor 2S globulin</td>
<td>Closely related to the 2S albumins</td>
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<tr>
<td><strong>Secondary structure</strong></td>
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<tr>
<td>11S globulin</td>
<td>7.8% α-helices 57.6% β-sheets 17.6% β-turns 16.9% random coils</td>
<td>13S globulin</td>
<td>16.0% α-helices 34.5% β-sheets 20.0% β-turns 14.4% random coils</td>
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<tr>
<td>7S Globulin</td>
<td>12.6% α-helices 49.6% β-sheets 12.2% β-turns 24.9% random coils</td>
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<tr>
<td>Globulin</td>
<td>31% α-helices 27% β-sheets 42% aperiodic structure</td>
<td>Globulin</td>
<td>25% α-helices 30% β-sheets 45% aperiodic structure</td>
</tr>
<tr>
<td>Albumin-2</td>
<td>16% α-helices 41% β-sheets 53% aperiodic structure</td>
<td>Albumin-2</td>
<td>14% α-helices 29% β-sheets 57% aperiodic structure</td>
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<tr>
<td><strong>Prolamins</strong></td>
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<td>Commons buckwheat:</td>
<td>Proteins with MM of 320 kDa and consisting of 6 similar subunits (52 to 59 kDa), which each consist of an acidic (30 to 40 kDa) and a basic (20 to 25 kDa) protein linked by an SS-bond.</td>
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Table 2–Continued.

<table>
<thead>
<tr>
<th>protein type</th>
<th>Amaranth</th>
<th>Buckwheat</th>
<th>Quinoa</th>
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<tr>
<td><strong>Proteins of amaranth, buckwheat, and quinoa</strong></td>
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<tr>
<td><strong>Amaranth</strong></td>
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<td><strong>Buckwheat</strong></td>
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<td><strong>Quinoa</strong></td>
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<td><strong>Amaranth Buckwheat</strong></td>
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<td><strong>Tartary buckwheat</strong></td>
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<td>Major protein with MM 15 and 17 kDa that were not impacted by a reducing agent. In addition, some minor proteins with a MM of 14 and 20 kDa and some SS-linked proteins with MM of 26 and 29 kDa have been observed.</td>
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<td><strong>Secondary structure</strong></td>
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<tr>
<td><strong>Glutelins</strong></td>
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<tr>
<td><strong>Common buckwheat</strong></td>
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<tr>
<td>SS-linked proteins with MM of 14 and 20 kDa and some SS-linked proteins with MM of 26 and 29 kDa have been observed.</td>
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<td><strong>Secondary structure</strong></td>
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</table>

References:
10. Marcone (1999); Quiroga and others (2010).
11. Radić and others (1996); Milisavljevic and others (2004); Choi and Ma (2006bb); Tang (2007aa).
13. Radić and others (1999); Bacs and others (2002).

**MM, molecular mass; SDS–PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SS, disulfide; ?, yet to be determined by future researchers.**

prolamins, and glutelins extracted from amaranth, buckwheat, and quinoa.

**Albumins**

**Amaranth.** Several authors (Konishi and others 1991; Gorinstein and others 2001; Drzewiecki and others 2003) have described 2 amaranth albumin types, which they obtained by a 2-step extraction procedure involving both water and a salt solution. A major protein fraction, correctly classified as albumin-1, is extracted from flour with salt solutions followed by dialysis against water and subsequent removal of the globulins (as pellet) by centrifugation. A minor protein fraction can then be extracted with water from the flour residue. The literature often refers to this fraction as “albumin-2,” “minor albumin,” or “globulin-P.” However, even if these proteins are extracted with water, salt is present in the flour as a result of the 1st extraction with salt solution. These proteins are thus most likely globulins, especially because they share several features with other amaranth globulins such as molecular size and surface reactivity (Drzewiecki and others 2003; Aphalo and others 2004; Quiroga and others 2007; Tandang-Silvas and others 2012). Already at low salt concentrations (≤0.01 M), the structure of the so-called albumin-2 evolves into a partially unfolded conformation, which may trigger aggregate formation (Castellani and others 1998). Based on tandem mass spectrometry of purified albumin-2 single proteins, of albumin-2 aggregates, and of partially purified globulin, Quiroga and others (2009) concluded that albumin-2 aggregates are isoforms of 11S globulin, indicating that albumin-2 and 11S globulin are encoded by the same gene. This way, amaranth albumin-2 is most likely a polymerization product of 11S globulin, which may explain its distinctive extractability in the Osborne fractionation process. Structural characteristics of the albumin-2 are discussed in the “Globulins” section.

For the analysis of amaranth albumins, classical sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) is preferred over chip electrophoresis, which compresses the bands of the very abundant proteins of low molecular mass (MM) (Džunková and others 2011). However, SDS–PAGE also has some limitations. For instance, the bands in an SDS–PAGE profile only
provide an estimation of the MM of a protein. Hence, the term apparent MM should be used in this context. The albumin-1 fraction in amaranth, extracted either with a salt solution followed by dialysis against water and removal of the globulins or simply extracted with water, has been frequently analyzed by SDS–PAGE. Most authors observed a major band corresponding to albumin-1 around 34 kDa (which withstands reducing conditions), and minor bands with apparent MMs in the range of 10 to 18 kDa (Gorinstein and others 1991b; Barba de la Rosa and others 1992a; Drzewiecki and others 2003). A high MM (apparent MM >78 kDa) protein has also been described (Gorinstein and others 2001; Barba de la Rosa and others 2009). Marcone and others (1994) described amaranth albumin-1 as a 133-kDa homo–dodecamer with 12, low–MM subunits held together by noncovalent (for example, hydrogen and hydrophobic) bonds. They found no evidence for covalent disulfide (SS) linkages between the subunits. According to Shewry (2016), the albumin-1 fraction migrates with a 2S sedimentation coefficient. If so, the homododecameric protein described by Marcone and others (1994) may be an aggregation product of the 2S albumin stabilized by noncovalent interactions. If not, it remains to be investigated whether the higher apparent MM bands observed by Barba de la Rosa and others (2009) and Gorinstein and others (2001) are derived from globulin impurities or whether some albums were not present in the albumin fraction purified by Marcone and others (1994). Based on SDS–PAGE, Segura–Nieto and others (1992) concluded that, of the Osborne protein fractions, albums are the most polymorphous. Analysis of amaranth albumin-1 by electrophoretic isoelectric focusing (IEF), electrophoretic titration, and zeta potential analysis indicated an isoelectric point (pI, the pH at which the net charge of the proteins equals zero) of 7.5 (Marcone and others 1994). Secondary structure analysis revealed that it has relatively high levels of β-sheets but a very little discernable tertiary structure, as evidenced by relatively low near-ultraviolet (UV) (240 to 320 nm) circular dichroic (CD) intensities (Marcone and others 1994). The secondary structure of amaranth albums has been estimated to consist of 4% α-helices, 37% β-sheets, and 55% aperiodic structure (Drzewiecki and others 2003).

**Buckwheat.** Most common buckwheat albums migrate with a 2S sedimentation coefficient and have apparent MMs between 8 and 12 kDa as determined by SDS–PAGE (Radovic and others 1999). A minor fraction of the 2S albums has an apparent MM close to 16 kDa. These proteins are not linked by SS-bonds (Radovic and others 1999). The 2S albums are widely distributed in dicotyledonous seeds. They are related to the 2S albums of wheat and inhibitors of trypsin and α-amylace from cereals (Kreis and others 1985). However, the 2S albums in legumes are present at higher levels and do contain SS-bonds (Shewry and Pandya 1999). The albums account for about 25% of total proteins extractable in salt solution, but this content is drastically reduced under sulfur-deficiency conditions during growth (Radovic and others 1999). Javornik and Kreft (1984) separated albums of common buckwheat by SDS–PAGE into 8 electrophoretic bands with apparent MMs between 17 and 67 kDa. Their results seem in line with those obtained for tartary buckwheat of which the nonreduced albumin fraction showed major bands at 64, 57, 52, 41, and 38 kDa (Guo and Yao 2006). Under reducing conditions, some minor bands at 14 to 22 kDa appeared at the expense of the 57 and 64 kDa albums, while the 38 and 41 kDa protein bands remained. The secondary structure of common buckwheat albums has been estimated to consist of 2% α-helices, 46% β-sheets, and 52% aperiodic structure (Drzewiecki and others 2003).

**Quinoa.** The major seed storage protein of quinoa is the 2S albumin. Under reducing conditions, an electrophoretically heterogeneous collection of proteins with apparent MMs between 8 and 9 kDa is observed (Brinegar and others 1996). Since native 2S albums in castor bean, rapeseed, and Brazil nut are dimers composed of a 7- to 9-kDa subunit SS linked to a 3- to 4-kDa subunit, it has been suggested that quinoa also contains the smaller 2S subunits, even if they are not resolved under the electrophoretic conditions employed (Brinegar and others 1996). Very different results were presented by Thanapornpoonpong and others (2008) when applying a fractionation scheme for amaranth proteins (Gorinstein and others 1991b). In their hands, it yielded 2 albumin fractions: the correctly classified albumin-1 and the alleged albumin-2 (cf. supra). When extracted from flour with salt solution followed by dialysis against water to precipitate the globulins, the albumin-1 is separated by SDS–PAGE into a wide range of protein subunits with apparent MMs between 25 and 83 kDa. The secondary structure of quinoa albums consists of 4% α-helices, 50% β-sheets, and 46% aperiodic structure (Drzewiecki and others 2003).

**Globulins**

**Amaranth.** The main amaranth protein is a globulin, the 11S globulin or amaratin. It consists of 3 subunits in their pro-form (proglobulin). Cotranslational signal peptide cleavage of the subunit in the endoplasmic reticulum induces association of the subunits in a head–to–tail pattern. The proglobulin is subsequently transported to the protein storage vacuoles. Herein, its post-translational cleavage by an endopeptidase releases acidic and basic chains and triggers the assembly of 2 trimers into a homohexamer (Tandang-Silvas and others 2012; Carrazo-Pena and others 2013). This homohexamer is made up of subunits with MMs between 52 and 59 kDa, each consisting of an acidic (34 to 36 kDa, pI 5.6) and a basic (22 to 24 kDa, pI 9.2) protein linked by an SS-bond (Konishi and others 1985; Barba de la Rosa and others 1992a; Barba de la Rosa and others 1992b; Valdez-Ortiz and others 2005). Martinez and others (1997) described the same or at least a very similar, protein, but falsely classified it as albumin-2 (see section “Albumins”), based on data by Konishi and others (1991). Much as the 11S globulin, this protein has an apparent MM of 300 kDa and contains subunits with apparent MMs of 52, 54, and 56 kDa. However, only the 52 and 56 kDa subunits seem to consist of a larger acidic peptide (31 to 38 kDa) SS-linked to a smaller basic (19 to 23 kDa) peptide. Gel filtration chromatography of amaranth proteins yielded 6 main fractions of around 300, 180, 120, 45, 25, and below 10 kDa (Zheleznov and others 1997; Juan and others 2007). The fraction with the highest apparent MM may correspond to the intact hexameric 11S globulin. We speculate that the fraction with apparent MM of 180 kDa represents 11S globulin trimers and/or the minor amaranth globulin. The smaller and larger proteins, as well as the 52 to 56 kDa subunits, were also detected by SDS–PAGE. The α- and β-peptides of 11S globulin appear in the fractions with apparent MMs of 45 and 25 kDa (Thanapornpoonpong and others 2008). Electrophoretic patterns under denaturing and reducing conditions showed 3 main fractions with apparent MMs between 50 and 64 kDa, 33 and 37 kDa, and 18 and 25 kDa, respectively (Juan and others 2007). Chip electrophoresis analysis of reduced amaranth globulins yielded the same 3 fractions (Dżunković and others 2011). Again, it was suggested that they represent the 52 to 56 kDa subunit and the constituting α- and β-peptides respectively, of the 11S globulin (Juan and others 2007). Marcone and Yada...
(1997) showed that 11S amaranth globulin is both glycosylated and phosphorylated.

The minor amaranth globulin, the 7S vicilin-like globulin, is a hetero-oligomer with an apparent MM estimated by gel filtration chromatography of 186 kDa made up of a variety of 8 noncovalently linked subunits (with SDS–PAGE MM of 90, 71, 40, 37, 35, 31, 24, and 16 kDa) (Marcone 1999). Barba de la Rosa and others (1992b) described a very similar protein containing 7 noncovalently linked subunits with MM of 72, 67, 52, 38, 34, 32, and 25 kDa. More recently, Quiroga and others (2010) reported on the amaranth 7S globulin subunit structure and observed main subunits with MM of 66, 52, 38, and 16 kDa, in reasonable agreement with Barba de la Rosa and others (1992b) and Marcone (1999). Electrophoretic IEF of the 7S amaranth globulin indicated a pI between 5.2 and 5.8. At lower pH, the 7S globulin was subjected to (i) a large surface charge density change and (ii) an acid-induced dissociation of its subunits (Marcone 1999). Near-UV (240 to 320 nm) CD intensities revealed that the purified 7S globulin secondary structure is relatively poor in α-helices, but possesses high levels of β-sheets (Marcone 1999). The secondary structure of the amaranth globulins has been estimated to consist of 31% α-helices, 27% β-sheets, and 42% aperiodic structure (Drzewiecki and others 2003), while that of the albumin–2 fraction, which most likely also contains globulins, has been estimated as 16% α-helices, 41% β-sheets, and 53% aperiodic structure (Tandang-Silvas and others 2012) and less hydrophobic surfaces than the albumins, as follows from 8-anilinonaphthalene-1-sulfonic acid fluorescence measurements (Gorinstein and others 2001).

**Buckwheat.** The major storage protein of common buckwheat is the 13S legume-like globulin. Konishi and others (1985) reported that its apparent MM is 440 kDa and that it is composed of at least 4 different subunit types (MMs of 18, 20, 32, and 36 kDa). It is now generally accepted that the 13S globulins exist as proteins with an apparent MM between 280 and 390 kDa (Maksimovic 1995; Marcone and others 1998; Fujino and others 1996). Separation of buckwheat globulin proteins in a sucrose gradient showed that they have apparent MMs of 52 to 679 kDa, the 2 largest proteins of which are no longer visible under reducing conditions. This indicates that they consist of SS-linked subunits. The same report mentions that reducing conditions did not induce notable changes in the 38 and 41 kDa proteins (Guo and Yao 2006). Drzewiecki and others (2003) reported 25% α-helices, 30% β-sheets, and 45% aperiodic structure for the common buckwheat globulin fraction, and 14% α-helices, 29% β-sheets, and 57% aperiodic structure for the so-called albumin–2 fraction, which probably also contains globulins.

**Quinoa.** The major seed storage protein of quinoa is the 11S globulin chenopodin, a hexameric protein with a MM of 320 kDa, of which each subunit consists of a basic (20 to 25 kDa) and an acidic (30 to 40 kDa) peptide covalently linked by an SS-bond (Brinegar and Goundan 1993; Thanapornpoonpong and others 2008). It makes up 37% of the total protein content in quinoa seeds (Abuogoch James 2009). SDS–PAGE, N-terminal sequencing, and amino acid analysis have allowed to conclude that it is a member of the highly conserved 11S storage globulin family (Brinegar and Goundan 1993; Balzotti 2006). The alleged albumin–2 fraction is also present in quinoa. It consists of 4 major subunits with apparent MMs of 23, 31, 35, and 52 kDa (Thanapornpoonpong and others 2008). All observed protein bands correspond to the subunits or monomers in the globulin fraction, which again indicates that the albumin–2 fraction, in fact, contains globulins. The secondary structure of quinoa globulin and the alleged albumin–2 fraction have been estimated to consist of 20% α-helices, 35% β-sheets, and 45% aperiodic structure and 10% α-helices, 37% β-sheets, and 53% aperiodic structure, respectively (Drzewiecki and others 2003).

**Prolamins**

A common observation for all pseudocereals is that they contain low levels of prolamins.

**Amaranth.** The prolamins of amaranth are made up of fewer and less abundant components than all other protein fractions (Segura-Nieto and others 1992). They cannot be readily analyzed by chip electrophoresis because of their low concentration (Džunková and others 2011). Nevertheless, SDS–PAGE under reducing conditions showed that they have apparent MMs of 52 to 54, 33 to 34, and 22 to 27 kDa and are linked by SS-bonds (Barba de la Rosa and others 1992a). They share common subunits with amaranth glutelins, and the differences in solubility between these proteins and amaranth prolamins have been ascribed to differences in levels and locations of SS-bonds (Barba de la Rosa and others 1992a).

**Buckwheat.** Skerritt (1986) separated a common buckwheat prolamin fraction by SDS–PAGE and observed a dominant range of low MM (10 to 28 kDa) proteins without sharp resolution and some minor bands at 32, 35, 68, 75, and 80 kDa. Naležec and others (2009) fractionated buckwheat prolamins into 5 two-dimensional (2D) SDS–PAGE subregions with proteins with apparent MMs around (i) 50 kDa and pls between 6.0 and 7.3, (ii) 39 kDa and pls between 6.2 and 6.8, (iii) 32 kDa and pls around 5.9, (iv) between 31 and 59 kDa and pls around 5.5, and (v) around 22 kDa and pls between 5.9 and 6.6. 2D electrophoresis is very useful for detecting buckwheat prolamins, especially since the lack of their sequences in databases makes it impossible...
to identify them directly with peptide-based mass spectrometry (Nałęcz and others 2009). Tarty buckwheat prolamin has 2 minor and 2 major protein bands at 14 and 20 kDa and 15 and 17 kDa, respectively, which are not influenced by a reducing agent. Under reducing conditions, 2 additional bands at 26 and 29 kDa are observed, possibly derived from the high MM proteins which do not enter the gel (Guo and Yao 2006).

**Quinoa.** The prolamin are an insignificant fraction of quinoa seed proteins (Fairbanks and others 1990; Thanapornpoonpong and others 2008) and information on their MM distribution is scarce.

**Glutelins**

**Amaranth.** 2D gel electrophoresis reveals some glutelins as major proteins in amaranth seeds (Segura-Nieto and others 1992). SDS–PAGE shows some proteins with low apparent MM (approximately 24 kDa), but more proteins of intermediate (32 to 38 kDa) and high apparent MM (48 to 57 kDa) (Gorinstein and others 1991b; Barba de la Rosa and others 1992a; Thanapornpoonpong and others 2008). Gorinstein and others (1991a) reported somewhat contradictory results. They found that the majority (83%) of the proteins of amaranth extracted in 55% (v/v) 2-propanol, containing 5% (v/v) mercaptoethanol, supposedly mostly are glutelins and have MMs around 10 to 14 kDa, that a 2nd fraction (7%) consists of proteins of 20 kDa, and that the remaining 10% is made up of minor fractions. Chip electrophoresis provided more detailed information. Of all the protein fractions, amaranth glutelins form the clearest patterns under reducing conditions with several clearly separated proteins which can be used to identify amaranth hybrid accessions and wild species (Džunková and others 2011). Glutelins of various amaranth species show polymorphism not only in the position of the bands in the patterns, but also in their intensity. Amaranth glutelins have 2 bands of medium intensity (15 and 17 kDa), 3 intense bands (21 to 23 kDa), 2 intense bands (31 and 33 kDa), 1 polymorphic low-intensity band (38 or 39 kDa) and a polymorphic area (50 to 65 kDa) (Džunková and others 2011). Part of the amaranth glutelin subunits (pL 5.7 to 6.3) show structural homology to the acidic globulin subunits (Vasco-Méndez and Paredes-López 1994).

**Buckwheat.** There is high glutelin polymorphism among different common buckwheat cultivars. Their glutelins consist of 3 to 5 SS-linked subunits with apparent MMs between 43 and 66 kDa (Gao and others 2008). Glutelins from tartary buckwheat in many cases only show minor and poorly defined SDS–PAGE bands. Some of these proteins do not enter the gel and/or are not soluble in the gel sample buffer (Guo and Yao 2006). Nevertheless, 9 electrophoretic subfractions with MMs ranging from 12 to 66 kDa, with little polymorphism, can be distinguished (Javornik and Kreft 1984; Guo and Yao 2006).

**Quinoa.** SDS–PAGE of quinoa glutelins yields few protein bands and great differences are noted between cultivars. Apparent MMs of the subunits range from 16 to 94 kDa (Thanapornpoonpong and others 2008). All proteins in the glutelin fraction have electrophoretic mobility identical to that of albumins and globulins (Fairbanks and others 1990).

**Denaturation and Aggregation Behavior**

In the studies cited below on protein denaturation and aggregation, the studied protein isolates were obtained by alkaline extraction and subsequent isoelectric precipitation (IEP) at acidic pH, unless otherwise stated. Table 3 lists the pH values of the extraction media, during IEP, and analysis of amaranth, buckwheat, and quinoa protein isolates.

**Protein denaturation as evaluated by differential scanning calorimetry**

**Amaranth.** Differential scanning calorimetry (DSC) of amaranth protein isolates revealed 2 endotherms with denaturation temperatures (T_d) readings in the ranges of 70 to 73 °C and 94 to 100 °C, respectively (Table 4). The 2nd endotherm was ascribed to denaturation of the alleged albumin-2 and globulins, whereas albumin-1, glutelins, and the minor components of globulins led to the 1st endotherm (Martínez and Anon 1996; Gorinstein and others 2001; Bejarano-Lujan and Netto 2010). A small decrease in T_d was noted when the extraction pH increased from 8.0 to 11.0. It should be stressed that T_d values of proteins may be influenced by specific prior extraction conditions since alkaline extraction can induce conformational changes (Martínez and Anon 1996; Castellani and others 1998; Salcedo-Chávez and others 2002; Abogoch James and others 2003; Cordero-De-Los-Santos and others 2005; Bejarano-Lujan and Netto 2010). Several authors (Ventureira and others 2012b; Shevkani and others 2014a; Shevkani and Singh 2015) reported similar T_d readings for such amaranth protein isolates. While 2 distinct denaturation peaks were visible at pH 8.0, no such peaks were observed at pH 2.0. This indicates that low pH induces extensive unfolding of amaranth proteins extracted under alkaline conditions (Abogoch James and others 2010; Ventureira and others 2012b; Bolontrade and others 2013a). Furthermore, Salcedo-Chávez and others (2002) noted that 10 different combinations of extraction pH and pH of IEP of amaranth protein isolates (Table 3) had no significant effect on their T_d readings. DSC of amaranth protein isolates obtained by extraction at pH 7.0 and subsequent precipitation by micellization revealed 2 endothermic transitions of which the T_d readings were 75.6 and 97.2, respectively (Cordero-De-Los-Santos and others 2005). Martinez and Anon (1996) reported on the T_d of amaranth protein fractions purified under relatively mild conditions. DSC T_d readings of 64 °C for albumin-1 and 94 °C for the falsely classified albumin-2 and most of the globulins were noted. The glutelin fraction showed endothermic peaks at 70 °C and 96 °C. Amaranth globulins also extracted under mild conditions showed endothermic transitions at 80.2 and 94.3 °C. These were attributed to the 7S and 11S globulins, respectively (Table 4) (Marcone 1999). The T_d of extracted proteins depends on pH. The so-called albumin-2 fraction has the highest T_d between pH 6.0 and 8.0. High-pressure treatment (200 to 600 MPa) also provokes denaturation of amaranth proteins. The protein fractions with the lower T_d readings (70 to 73 °C range, see above) have greater sensitivity to high-pressure treatment than proteins characterized by T_d readings in a 94 to 100 °C range. Whereas the proteins with lower T_d are almost completely denatured after treatment at 200 MPa, those of higher thermal stability partially retain their native structure. Furthermore, the proteins with low and high T_d that resist high-pressure treatment have lower and higher thermal stability, respectively, than the untreated protein fractions (Condes and others 2012; Condes and others 2015).

**Buckwheat.** Common buckwheat proteins show a minor and a major denaturation peak at about 80 °C and about 102 °C, which have been ascribed to the 8S and 13S globulins, respectively (Table 4) (Tang 2007a). The T_d values were not affected by prior lipid extraction, indicating that endogenous lipids do not affect the thermal stability of buckwheat proteins (Tang 2007a). Extrem acidic and alkaline pH conditions induce a decrease in T_d of the
### Table 3—Overview of pH of extraction, pH of isoelectric precipitation, and pH of analysis reported for protein isolates used in studies on denaturation, aggregation, and solubility as well as foaming, emulsifying, and gelling properties of amaranth, buckwheat, and quinoa proteins.

<table>
<thead>
<tr>
<th>Pseudocereal</th>
<th>pH of extraction</th>
<th>pH of IEP</th>
<th>pH of analysis</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Protein denaturation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amaranth</td>
<td>8.0 to 11.0</td>
<td>5.0</td>
<td>7.0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>9.0</td>
<td>3.0 to 7.0</td>
<td>7.0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>9.0 and 11.0</td>
<td>5.0</td>
<td>7.0</td>
<td>3, 4, 5, 6, 7</td>
</tr>
<tr>
<td></td>
<td>9.0</td>
<td>5.0</td>
<td>7.0</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>4.5</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.0</td>
<td>4.5</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.5</td>
<td>4.5</td>
<td>4.5</td>
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<td>5.7</td>
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<td>5.0</td>
<td>5.0</td>
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<td>8.5</td>
<td>5.0</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.0</td>
<td>–</td>
<td>7.0</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>11.0</td>
<td>5.0</td>
<td>7.0</td>
<td>10, 11</td>
</tr>
<tr>
<td>Buckwheat</td>
<td>8.0</td>
<td>4.5</td>
<td>7.0</td>
<td>9</td>
</tr>
<tr>
<td>Quinoa</td>
<td>9.0 and 11.0</td>
<td>5.0</td>
<td>7.0</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>9.0</td>
<td>5.0</td>
<td>7.0</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>8.0 to 11.0</td>
<td>4.5</td>
<td>7.0</td>
<td>15</td>
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<tr>
<td><strong>Protein aggregation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amaranth</td>
<td>9.0</td>
<td>5.0</td>
<td>7.0</td>
<td>4, 16</td>
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<tr>
<td></td>
<td>9.0 to 11.0</td>
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<td>7.0</td>
<td>17</td>
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<td></td>
<td>11.0</td>
<td>5.0</td>
<td>7.0</td>
<td>10, 11, 18</td>
</tr>
<tr>
<td>Quinoa</td>
<td>8.0 to 12.0</td>
<td>–</td>
<td>8.0 to 12.0</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>9.0</td>
<td>5.0</td>
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<td>20, 21</td>
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<tr>
<td><strong>Solubility</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amaranth</td>
<td>0.25% NaOH&lt;sup&gt;a&lt;/sup&gt;</td>
<td>–</td>
<td>3.0 to 9.0</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>9.0</td>
<td>5.0</td>
<td>7.0 and 8.5</td>
<td>23</td>
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<tr>
<td></td>
<td>9.0</td>
<td>5.0</td>
<td>7.0</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>9.0</td>
<td>–</td>
<td>3.0, 4.5, and 7.0</td>
<td>9</td>
</tr>
<tr>
<td>Buckwheat</td>
<td>0.25% NaOH&lt;sup&gt;a&lt;/sup&gt;</td>
<td>–</td>
<td>3.0 to 9.0</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>4.5</td>
<td>7.0</td>
<td>24, 25</td>
</tr>
<tr>
<td>Quinoa</td>
<td>0.015 M NaOH&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.5</td>
<td>3.0 to 8.0</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>9.0 and 11.0</td>
<td>5.0</td>
<td>3.0 to 11.0</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>5.0 to 10.0</td>
<td>3.0 to 6.0</td>
<td>1.0 to 10.0</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>9.0</td>
<td>5.0</td>
<td>3.0 to 11.0</td>
<td>14</td>
</tr>
<tr>
<td><strong>Foaming properties</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amaranth</td>
<td>0.25% NaOH&lt;sup&gt;a&lt;/sup&gt;</td>
<td>–</td>
<td>7.0</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>9.0 and 11.0</td>
<td>5.0</td>
<td>4.0, 7.0, and 9.0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>9.0</td>
<td>5.0</td>
<td>7.5</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>9.0</td>
<td>5.0</td>
<td>2.0 and 8.0</td>
<td>4, 29</td>
</tr>
<tr>
<td></td>
<td>9.0</td>
<td>5.0</td>
<td>2.0 to 9.0</td>
<td>5</td>
</tr>
<tr>
<td>Buckwheat</td>
<td>0.25% NaOH&lt;sup&gt;a&lt;/sup&gt;</td>
<td>–</td>
<td>7.0</td>
<td>22</td>
</tr>
<tr>
<td>Quinoa</td>
<td>0.015 M NaOH&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.7</td>
<td>7.0</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>0.015 M NaOH&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.5</td>
<td>3.0 to 8.0</td>
<td>26</td>
</tr>
<tr>
<td><strong>Emulsifying properties</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amaranth</td>
<td>–</td>
<td>–</td>
<td>7.0</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>9.0</td>
<td>5.0</td>
<td>2.0, 6.3, and 8.0</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>9.0</td>
<td>5.0</td>
<td>2.0 and 8.0</td>
<td>3</td>
</tr>
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<td></td>
<td>9.0</td>
<td>5.0</td>
<td>2.0 to 9.0</td>
<td>5</td>
</tr>
<tr>
<td>Buckwheat</td>
<td>–</td>
<td>–</td>
<td>7.0</td>
<td>22</td>
</tr>
<tr>
<td>Quinoa</td>
<td>0.015 M NaOH&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.7</td>
<td>7.0</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>0.015 M NaOH&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.5</td>
<td>3.0 to 8.0</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>5.0 to 10.0</td>
<td>3.0 to 6.0</td>
<td>10.0</td>
<td>27</td>
</tr>
<tr>
<td><strong>Gelling properties</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amaranth</td>
<td>9.0</td>
<td>4.5</td>
<td>7.0</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>9.0</td>
<td>5.0</td>
<td>7.0</td>
<td>35, 36</td>
</tr>
<tr>
<td>Quinoa</td>
<td>9.0</td>
<td>5.0</td>
<td>8.5 and 10.5</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>8.0 to 11.0</td>
<td>4.5</td>
<td>6.5</td>
<td>15</td>
</tr>
</tbody>
</table>

*a*The authors mention NaOH concentration instead of pH.

<sup>–</sup>“—” Indicates not specified.


IEP, isoelectric precipitation.
Table 4—Differential scanning calorimetry (DSC) protein denaturation temperatures reported for Osborne fractions from amaranth, common and tartary buckwheat, and quinoa.

<table>
<thead>
<tr>
<th>Protein fraction</th>
<th>Reference</th>
<th>Denaturation temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin-1, minor components of globulins, and glutenins</td>
<td>Martinez and Anon (1996)</td>
<td>70 to 73</td>
</tr>
<tr>
<td>Albumin-2, and 11S globulin</td>
<td>Marcone (1999)</td>
<td>94 to 100</td>
</tr>
<tr>
<td>7S globulin</td>
<td>Tang (2007a)</td>
<td>80.2</td>
</tr>
<tr>
<td>85 globulin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>102</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13S globulin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11S globulin</td>
<td>Abugoch James and others (2009)</td>
<td>98 to 99</td>
</tr>
</tbody>
</table>

DSC, differential scanning calorimetry; $T_d$, denaturation temperature; ?, yet to be determined by future researchers.

The buckwheat globulin fraction, thus indicating lower protein thermal stability (Choi and Ma 2005).

**Quinoa.** Heat-induced denaturation of quinoa proteins has only sporadically been investigated. Quinoa protein isolate denatures at about 98 °C (Abugoch James and others 2008; Steffolani and others 2015; Ruiz and others 2016). Protein isolates extracted at pH 11.0 did not show any endotherm, indicating that such alkaline extraction resulted in complete denaturation (Abugoch James and others 2008; Ruiz and others 2016). Gorinstein and others (1996) reported a $T_d$ of 58 °C for quinoa globulins. However, the main quinoa globulin, namely the 11S globulin (see section “Globulins”), and 11S globulins of other protein sources (such as soy) typically denature at much higher temperatures (> 85 °C) (Martinez and Anon 1996; Tarone and others 2013). Abugoch James and others (2009) reported a $T_d$ of 99 °C for quinoa proteins in flour (Table 4). Since quinoa starch gelatinizes at around 60 °C (Li and others 2016), it cannot be excluded that the gelatinization endotherm overlaps with an endotherm of proteins with lower $T_d$.

**Effect of salt, reducing agents, and protein denaturants.** Other constituents present can affect protein denaturation. Salt increases the thermal stability of amaranth proteins (Bolontrade and others 2013a), amaranth albumin-2 (Castellani and others 1998), and buckwheat globulins (Choi and Ma 2005; Tang 2007a), as evidenced by higher $T_d$ readings. At ionic strength of 0.54 M, the $T_d$ of amaranth albumin-2 reaches a maximum at pH 6.0, but it is much lower below pH 5.0 and above pH 8.5. Thus, both high and alkaline pH conditions induce conformational changes in its structure, which lower its thermal stability. Furthermore, at alkaline pH values and ionic strength of 0.54 M, a 2nd endotherm appears at about 73 °C. This points to the formation of new molecules of lower thermal stability than that of the undivided albumin-2 ($T_d$ of 102 °C, pH 7.5). In this context, sedimentation and gel filtration elution patterns have shown that alkaline pH triggers the dissociation of the so-called albumin-2 into its trimeric subunits (see sections “Albumins” and “Globulins”). These subunits are probably less thermostable than the undivided molecules (Castellani and others 1998). The $T_d$ of common buckwheat globulins depends on the type of salt and decreases in the lyotropic series of anions (thus in the order Cl$^-$, Br$^-$, I$^-$, and SCN$^-$) (Choi and Ma 2005; Tang 2007a). While Cl$^-$ and Br$^-$ ions stabilize the protein, I$^-$ and SCN$^-$ ions destabilize it (Tang 2007a). The presence of the protein denaturant urea led to a decrease in $T_d$ of amaranth globulins, whereas 2 mercaptoethanol, an SS-reducing agent, did not affect it (Gorinstein and others 1996; Bolontrade and others 2013a). Hence, hydrogen bonds are more important for the thermal stability of amaranth globulins than SS-bonds (Gorinstein and others 1991b; Gorinstein and others 1996). Much as it is the case for amaranth proteins, the $T_d$ readings of common buckwheat globulins decrease with increasing levels of protein denaturants (including urea and SDS), confirming the importance of hydrogen bonds and hydrophobic interactions for their thermal stability (Tang 2007a). It is of note here that sufficiently high concentrations of 2-mercaptoethanol are necessary to decrease the $T_d$ of buckwheat 13S globulin (Choi and Ma 2005; Tang 2007c).

**Protein aggregation**

Little information is available on heat-induced aggregation of pseudocereal proteins, especially so for proteins extracted under mild conditions.

**Amaranth.** Thermal treatment (10 min at 90 °C) of amaranth proteins suspended in a potassium phosphate buffer (pH 8.5) causes a decrease in protein solubility. This is mainly due to aggregation of the globulin fraction (Scilingo and others 2002). Moderate heating (50 °C) of amaranth proteins during alkaline extraction induces partial protein unfolding and, as a consequence, the formation of high MM aggregates, as evidenced by DSC, size-exclusion high-performance liquid chromatography (SE-HPLC), a lower free thiol (SH) content, and a reduction in protein solubility (Bejarano-Lujan and Netto 2010). Mild heating (70 °C) of aqueous dispersions of amaranth proteins decreases their surface hydrophobicity. The effect of longer heating times and higher temperatures on surface hydrophobicity depends on the pH (9.0 compared with 11.0) at which the proteins are extracted. Both protein denaturation and aggregation likely affect their surface hydrophobicity. High MM aggregates are formed during heating, which are stabilized by both SS-bonds and noncovalent bonds. A protein isolate obtained at pH 9.0 is more sensitive to thermal treatment than one obtained at pH 11.0 (Avanza and Anon 2007; Condes and others 2013).
High-pressure treatment of amaranth protein isolates affects the degree of dissociation or association of amaranth proteins obtained by alkaline isolation. High-pressure treatment induces the formation of insoluble aggregates, triggers the dissociation of soluble aggregates, and increases surface hydrophobicity (Condes and others 2012, 2015).

Protein aggregation can also occur at room temperature when altering the pH and/or ionic strength of the solution, which evidently affects protein solubility (see section “Solubility”). Higher ionic strength in a pronounced way reduces the solubility of amaranth proteins, especially for proteins 1st isolated at pH 9.0 and subsequently dispersed in a pH 2.0 buffer, indicating the importance of charge-shielding for protein aggregation and subsequent precipitation (Bolontrade and others 2013a).

**Buckwheat.** DSC, SDS–PAGE, and SE–HPLC analyses have shown that heat treatment of common buckwheat globulin dissolved in 10.0 mM phosphate buffer (pH 7.4) containing 1.0 M NaCl induces (i) the dissociation of proteins with apparent MM in a 34 to 36 kDa and a 21 to 22 kDa range and (ii) association of proteins with subsequent aggregate formation (Choi and Ma 2006a; Choi and others 2006). During thermal aggregation, intermolecular SS-bonds are formed (Choi and others 2006). In the presence of N-ethylmaleimide, an SH-blocking agent, aggregates of higher MM and hydrodynamic radius are formed than in its absence (Choi and Ma 2006a). Transmission electron microscopy demonstrated the formation of strand-like small aggregates as well as that of large compact globular soluble macroggregates (Choi and Ma 2006a; Choi and others 2006). Protein aggregation also occurs during puffing (Mariotti and others 2008) or autoclave treatment (Tomotake and others 2012) of dehulled common buckwheat seeds. Puffing reduces the surface hydrophobicity of common buckwheat flour proteins. Protein aggregation presumably makes its surfaces less accessible for the hydrophobic fluorescent probe used in the analysis due to increasing mutual interactions between hydrophobic protein surfaces (Mariotti and others 2008).

**Quinoa.** The effect of heat treatment (0 to 15 min, 100 °C) on quinoa proteins isolated at pH 9.0 depends on the pH during heating (Mäkinen and others 2015, 2016). Heating at pH 8.5 or 10.5 disrupts the SS-bond connecting the acidic and basic subunits of 11S globulin (Table 2), while the covalent bond remains intact when heating is at pH 6.5. Large aggregates with intermolecular SS-bonds are formed by heating at pH 6.5 and 8.5, while no aggregates are formed at pH 10.5, which are extractable in SDS-containing medium. The results also indicate that a low degree of protein hydrolysis occurs during heating at pH 10.5 (Mäkinen and others 2016). The free SH-content of the globulin fraction increases during the 1st 5 min of heating at pH 8.5 and 10.5, but it decreases with prolonged heating to a level lower than that of the nonheat-treated globulin fraction. Heating at pH 6.5 results in a slight increase in the level of free SH. The surface hydrophobicity increased during the 1st 5 min of heating (100 °C) at all tested pH values. Upon prolonged heating, a small increase in surface hydrophobicity was observed at pH 6.5 and 8.5, while at pH 10.5 it decreased almost to the reading observed for the nonheat-treated globulins (Mäkinen and others 2016). Heating of quinoa proteins obtained under alkaline conditions (pH 8.5 or pH 10.5) may trigger deamidation of glutamine and asparagine residues and thereby drastically affect protein surface properties. The degree of deamidation after heating was about 30% at both pH 8.5 and 10.5 (Mäkinen and others 2015).

**Techno-functional Properties**

The techno–functional properties of amaranth proteins have been more extensively studied than those of their buckwheat and quinoa counterparts. As above, protein isolates in the studies described below were obtained by alkaline extraction and subsequent IEP at lower pH (Table 3), unless otherwise stated. We again stress that the techno–functional properties of the extracted proteins are likely impacted by the specific extraction conditions since these can trigger conformational changes.

**Solubility**

**Protein isolates.** Protein solubility is important for its techno–functional properties in different (food) applications. In general, the solubility of amaranth, buckwheat, and quinoa proteins is poor (range of 2 to 35% range) in a pH ranging from 3.0 to 5.0. Maximum solubility, with percentages ranging from 50 to almost 100%, is found at alkaline pH values (Bejosano and Corke 1999; Tomotake and others 2002; Aluko and Monu 2003; Silva-Sanchez and others 2004; Cordero-De-Los-Santos and others 2005; Tömösközi and others 2008; Tang and others 2009; Bejarano-Lujan and Netto 2010; Eshohaimy and others 2015; Steffolani and others 2015; Ruiz and others 2016). Depending on the particular study, very good solubility has also been observed at pH 2.0 (Tomotake and others 2002; Tang and others 2009; Shevkani and others 2014a). Part of the differences in optimal pH values and solubility percentages can be explained by differences in cultivar (Silva-Sanchez and others 2004; Shevkani and others 2014a; Steffolani and others 2015) and extraction procedure (such as the extraction pH). However, the latter has only been demonstrated for quinoa proteins. In this case, proteins extracted at pH 9.0 are more soluble at pH values exceeding 5.0 than those extracted at pH 11.0 (Abogoch James and others 2008; Ruiz and others 2016). Finally, no significant differences in solubility were observed between amaranth, buckwheat, or quinoa proteins over a pH range of 3.0 to 11.0. Hydrolysis of amaranth proteins with trypsin (Condes and others 2009), of amaranth and buckwheat proteins with pepsin (Bejosano and Corke 1999), and of quinoa proteins with Alcalase (Aluko and Monu 2003) improved solubility. The solubility of quinoa protein hydrolysates (degree of hydrolysis [DH] of 48%) hardly depends on pH, in contrast to what has been observed for their nonhydrolyzed counterparts (Aluko and Monu 2003).

**Osborne-type fractions.** Amaranth albumins and globulins and proteins extracted under alkaline conditions show similar low solubility at pH values between 3.0 and 5.0. Above pH 5.0, its albumins (maximum solubility of 90% at pH 11.0) are more soluble than its globulins (maximum solubility around 50% at pH 11.0) (Tömösközi and others 2008). According to Carrazo-Pena and others (2013), the amaranth 11S globulins, which are the major proteins of amaranth (Table 2), show minimal solubility between pH 2.6 and 4.6 (around 10% and 20% at low and high ionic strength, respectively). Approximately 90% of the 11S globulin fraction is soluble at pH values exceeding 6.1. The introduction of 4 successive methionine residues in the variable region V of recombinant amaranth 11S globulin drastically reduces its solubility due to an increase in surface hydrophobicity. Here, it is of benefit to mention that the properties of the recombinant unmodified 11S globulin differ from those of the native protein purified from amaranth seeds, most likely due to the lack of co- and/or post-translational cleavages of the recombinant unmodified protein (see section “Globulins”) (Carrazo-Pena and others 2013).
Foaming properties

Protein isolates. The pH affects both amaranth protein foam formation and stability. At pH 2.0, amaranth proteins foam better than at pH 8.0 due to faster diffusion to and/or faster adsorption at the air/water interface. The higher flexibility of amaranth proteins at acid than at alkaline pH facilitates their diffusion to and adsorption at the air/water interface, thereby reducing the interfacial tension (Bolontrade and others 2013a). Ventureira and others (2012a) further showed that acid-treated amaranth extract contains an endogenous peptidase which is active at pH 2.0. Partial hydrolysis of amaranth proteins into smaller fragments improves their diffusion to the air/water interface. Amaranth protein foams are more stable at pH 2.0 than at pH 8.0, due to their interfacial protein films being more viscoelastic and flexible at the former pH (Bolontrade and others 2013b). However, the pH at which foaming is optimal seems to be cultivar-dependent (Shevkani and others 2014a; Steffolani and others 2015). In addition, differences in foaming may also be explained by differences in protein extraction procedures. Abugoch James and others (2010) showed that whether amaranth proteins are isolated from flour at pH 9.0 or 11.0 influences their conformation and surface hydrophobicity and, as a consequence, their foaming properties. While their foaming capacity was similar, foams prepared from extracts obtained at pH 11.0 were more stable than those from extracts obtained at pH 9.0, especially under acidic conditions (Abugoch James and others 2010). High ionic strength favors amaranth protein foam formation at pH 2.0 and pH 8.0 (Bolontrade and others 2013a). However, the obtained foams are less stable than those produced at low ionic strength (Bolontrade and others 2013b). At low ionic strength, the protein film at the interface is thicker. This retards gas diffusion and, hence, bubble disproportionation and coalescence. Furthermore, there is more electrostatic repulsion at low than at high ionic strength (Bolontrade and others 2013b). Amaranth proteins foam better than soy protein isolates obtained under similar conditions, due to a faster reduction in interfacial tension in the former case (Bejosano and Corke 1999; Ventureira and others 2012a). Bejosano and Corke (1999) reported better stability of amaranth than of soy protein foams, while Ventureira and others (2012a) observed amaranth protein foams to have lower stability than those of soy proteins. Buckwheat (Bejosano and Corke 1999) and quinoa (Aluko and Monu 2003) protein isolates show optimal foaming properties at higher pH than do amaranth proteins.

Osborne-type fractions. Papers dealing with foaming of proteins isolated in a mild way are rather limited. Amaranth albumins foam better and yield foams with better stability than amaranth proteins extracted under alkaline conditions. The latter, in turn, foam better than amaranth globulins (Tömősközi and others 2008). The foaming properties of amaranth albumins are even similar to those of casein (Tömősközi and others 2008) and egg albumins at acidic pH values (Silva-Sanchez and others 2004). Both protein types are frequently used in industrial applications for their foaming behavior.

Effect of (pre)treatments. (Pre)treatments of pseudocereal protein isolates have been applied for improving their foaming properties. Amaranth proteins 1st treated with acid (pH 2.0 for 3 h) and then neutralized to pH 7.5 reduce the interfacial tension faster than their untreated counterparts. This results in improved foam formation and stability. The acid-treated proteins are partially unfolded and undergo limited hydrolysis due to the presence of an endogenous peptidase (see above). Limited hydrolysis by the endogenous peptidase thus favors migration of proteins to the air/water interface and renders them more flexible, thereby enhancing conformational rearrangements at the interface (Ventureira and others 2012a). Protein hydrolysis is indeed a frequently used pretreatment to improve foam formation (Bejosano and Corke 1999; Aluko and Monu 2003; Condé and others 2009). Limited hydrolysis of amaranth proteins (DH of 6.4%) results in foams that are more stable than those produced with their nonhydrolyzed counterparts, while foams prepared from moderately hydrolyzed amaranth (DH varying between 11.6% and 17.0%) or buckwheat (DH of 11.8%) (Bejosano and Corke 1999) proteins, or from severely hydrolyzed quinoa (DH of 48%) (Aluko and Monu 2003) proteins, are less stable than those from their native counterparts. The latter proteins are probably too small to form viscoelastic interfacial layers with sufficient mechanical strength.

Effect of endogenous constituents. Other constituents in pseudocereal protein isolates can also affect foaming properties. For amaranth, Fidantsi and Doxastakis (2001) found that interactions between proteins and polysaccharides can enhance both foam formation and stabilization. Prior defatting of amaranth flour has, depending on the cultivar, no or a positive impact on foam formation, while foam stabilization clearly improves as a result of flour-defatting (Shevkani and others 2014b). Mixed protein-lipid interfaces are inherently unstable as they compete for the air/water interface and weaken each other’s ability to stabilize it (Wilde 2000). Depending on the cultivar, quinoa may contain saponins, which are a complex mixture of triterpene glycosides with surface-active properties. Removing saponins from alkali-extracted proteins reduces foam formation, but makes the obtained foams more stable (Chauhan and others 1999).

Emulsifying properties

Protein isolates. Similar to what is described above for foaming properties (see section “Foaming properties”), the pH also affects emulsification and stabilization by amaranth proteins. In general, emulsification by amaranth protein isolates is low at their pI, as under such conditions proteins cannot diffuse rapidly to the interface (Shevkani and others 2014a). While Bejosano and Corke (1999) reported better emulsifying properties for amaranth than for soy proteins, Cordero-De-Los-Santos and others (2005) and Shevkani and others (2014a) found both protein types to behave similarly in this respect. In addition, amaranth proteins obtained via alkaline extraction and subsequent IEP produce a more stable emulsion than do proteins obtained by alkaline extraction at the same pH and subsequently dialyzed against water. This is likely due to a lower coalescence rate as shown by monitoring the oil droplet size distribution with light microscopy (Fidantsi and Doxastakis 2001). The emulsion stability between pH 7.0 and 10.0 of buckwheat proteins is lower than observed for soy protein isolate and casein (Tomotake and others 2002). In contrast, while Bejosano and Corke (1999) reported that commercial soy protein isolates have emulsifying properties inferior to those of buckwheat proteins, Zheng and others (1997) reported both protein emulsions to behave in a similar way. In addition, below pH 4.0 and above pH 6.0, emulsification and stability of spray-dried buckwheat protein emulsions are comparable to those formed from spray-dried soy protein isolates (Tang 2007b). With regard to quinoa protein emulsions, Elshahamy and others (2015) studied the effect of protein concentration (0.1% to 3.0%). At higher protein concentrations, emulsification increases, but the obtained emulsions are less stable.

Osborne-type fractions. Amaranth albumins show optimal emulsification around pH 5.0 (Silva-Sanchez and others 2004). At pH 8.0, Tömősközi and others (2008) reported superior emulsifying properties for amaranth albumins than for its globulins.
However, the purity of the albumin fraction was much lower than that of the globulins (49.0 compared with 87.0%). It may have been contaminated with polysaccharides, which positively affect both the formation and stability of interfaces (Patino and Pilosof 2011). The overall emulsification by amaranth globulins is strongly pH-dependent. Emulsification and stabilization are poor at pH < 5.0, but they improve substantially between pH 5.0 and 7.0 (Konishi and Yoshimoto 1989; Marcone and Kakuda 1999). Under mildly alkaline conditions, they are inferior to those measured at pH > 9.0 (Konishi and Yoshimoto 1989). Between pH 3.0 and 9.0, amaranth globulins show better emulsification than their soy counterparts (Marcone and Kakuda 1999). This may be due to the high degree of glycosylation and phosphorylation of amaranth globulins (see section “Globulins”). Furthermore, the protein-stabilizing layer, which encloses the oil droplets, is highly charged when the amaranth globulins are further from their pl, which may enhance charge repulsion between emulsion particles. In addition, high levels of glycosylation and phosphorylation also generate a more hydrated layer, which retards droplet coalescence (Marcone and Kakuda 1999). At pH 7.0 or 8.0, the emulsification by amaranth globulins is lower than that by casein protein isolate (Konishi and Yoshimoto 1989; Tömösközi and others 2008).

**Effect of (pre)treatments.** The emulsifying properties of pseudocereal protein isolates can be improved by (pre)treatments. Heat treatment of a solution of amaranth globulins (50 to 100 °C) at pH 7.5 improves emulsification due to (partial) denaturation of the globulins (Table 3), which goes in hand with the exposure of hydrophobic groups, as evidenced by 8-anilinonaphthalene-1-sulfonic acid fluorescence measurements (Konishi and Yoshimoto 1989). Acid treatment (pH 2.0 for 3 h) and subsequent neutralization to pH 7.5 of amaranth proteins obtained under alkaline conditions improves their adsorption kinetics to and the elasticity of the oil/water interface obtained. CD intensities and DSC data have revealed that acid treatment induces extensive unfolding of amaranth proteins. This may stabilize the interface. Moreover, higher elasticity of the interfacial oil/water layer results in higher resistance of the prepared emulsions against coalescence and, hence, in improved emulsion stability (Ventureira and others 2012b). At a pH of 2.0, enzymatic hydrolysis of amaranth proteins with trypsin to a DH of 2.2% or with Alcalase to DHs of 1.7 or 9.5% slightly improves their emulsification (Ventureira and others 2010). Severe enzymatic hydrolysis of quinoa proteins with Alcalase (DH of 48%) lowers emulsification between pH 3.0 and 8.0, but improves the stability of the formed emulsion (Aluko and Monu 2003). This indicates that some peptides were still large enough to form a stable viscoelastic layer at the oil/water interface.

**Effect of endogenous constituents.** Other constituents in pseudocereal protein isolates can also affect emulsifying properties. Polysaccharides contribute to emulsion stability by crosslinking proteins that are adsorbed at the interface (Fidantsi and Doxastakis 2001). Removing saponins from alkaline protein extracts of quinoa reduces their emulsification, while the obtained emulsions are more stable (Chauhan and others 1999). The presence of lipids in buckwheat protein isolates negatively affects the protein emulsifying properties, especially at pH > 6.0 (Tang 2007c). As observed for foaming (see section “Foaming properties”), mixed protein-lipid interfaces negatively affect the emulsifying properties, as proteins and lipids interfere with each other in the stabilization of the interface (Wilde 2000).

**Gelling properties**

Although protein gelation is important in food processing, the literature on pseudocereal protein gelation is scarce and limited to a few studies on amaranth and quinoa proteins.

**Protein isolates.** Avanza and others (2005a) found a protein concentration of 7.0% (w/v) and a temperature of 70 °C to be the minimal requirements to induce gel formation by amaranth proteins. At high protein concentrations, amaranth proteins form ordered gel structures, which are stabilized by intramolecular SS-bonds formed as a result of SH/SS-interchange reactions between denatured 11S globulins and the alleged albumin-2 (see section “Albumins”). Noncovalent bonds (primarily hydrogen bonds and hydrophobic interactions), mainly between monomeric proteins of apparent MM 42 kDa, stabilize the gel structure, but to a lower extent than do SS-bonds. Proteins with an apparent MM below 20 kDa are present in the interstitial spaces of the gel matrix (Avanza and others 2005b), which is elastic, hard, and displays high fracturability and cohesiveness (Avanza and others 2005a). At lower protein concentrations (< 15% w/v), temperatures close to 70 °C and short heating times, more disordered gel structures are formed in which the proportion of noncovalent bonds is higher than in gels formed at high protein concentration, higher temperatures, or longer heating times (Avanza and others 2005a, 2005b).

Whether amaranth proteins are isolated under acid or alkaline conditions has a strong impact on the structure and rheology of the formed gels. Gels from proteins extracted at pH 4.5 are inferior to those formed from proteins extracted at pH 9.0 due to lower globulin levels in the former extract (Bejarano-Lujan and others 2010). Higher temperatures during extraction at pH 9.0 further improve amaranth protein gelation. This likely results from partial protein denaturation and aggregation (Bejarano-Lujan and others 2010).

Gels prepared from quinoa proteins isolated under alkaline conditions and heated (15 min at 100 °C) at pH 10.5 are more elastic and show a finer and more regular structure than when produced by heating at pH 8.5. Heating at pH 10.5 forms small soluble aggregates, while at pH 8.5 it produces larger aggregates, which then leads to coarse gel structures (Mäkinen and others 2015). Very recently, Ruiz and others (2016) demonstrated that the (alkaline) pH at which quinoa proteins are isolated has a strong impact on their gelation. Proteins isolated at pH 8.0 or 9.0 (followed by IEP at pH 4.5) formed dense semisolid gels already during heating (from 20 to 90 °C at 1 °C/min), while under such conditions no gel was formed from proteins isolated at pH 10.0 or 11.0. A soft gel was formed only during cooling (from 90 to 20 °C at 3 °C/min) when quinoa protein extraction was done at pH 10.0 or 11.0. More protein is denatured during extraction under very alkaline conditions, which likely results in aggregation and sedimentation of large particles and reduced aggregation of smaller particles.

**Osborne-type fractions.** In contrast to gel formation by amaranth protein isolates (see above), native amaranth 11S globulin forms ordered gels when its concentration exceeds 21.8% and when heat-treated at a temperature of at least 100 °C (Carrasco-Pena and others 2013). The introduction of 4 methionine residues in the variable region V of the 11S amaranth progglobulin decreases the elastic (G’’) and viscous (G’’) moduli of the obtained gel. Here again, differences between gelling properties of native and recombinant 11S globulins are ascribed to the lack of co- and/or post-translational cleavages of the recombinant protein (see section “Globulins”) (Carrasco-Pena and others 2013).
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cysteine residues present in their proteins (D’Amico and others 2015). As illustrated in the section “Denaturation and aggregation behavior,” high-temperature treatment provokes the denaturation of amaranth, buckwheat, and quinoa proteins and, as a consequence, triggers the formation of high MM aggregates stabilized by intermolecular SS-bonds. Hatcher and others (2011) studied textural attributes of soja noodles [that is, noodles that are commonly made from a blend of wheat flour and at least 60% buckwheat flour and water (Guménez-Bastida and others 2015)] made from 80% whole meal buckwheat from different cultivars. The inclusion of dehulled, whole meal brown Tartary buckwheat resulted in noodles of improved texture compared to those produced from whole meal green testa or from common buckwheat flour (Hatcher and others 2011). The incorporation of quinoa flour (16, 50, 100, 150, and 184 g/kg flour basis) in a maize gluten-free formulation resulted in a product which, when cooked, had acceptable stickiness (Caperuto and others 2001). In addition, inclusion of whole meal quinoa in an amaranth spaghetti recipe showed beneficial effects on overall cooking quality and texture parameters (Chillo and others 2008b).

Wheat and gluten-free cookies

Only few studies deal with the incorporation of amaranth or quinoa in cookie systems. Sindhuja and others (2005) included increasing levels of whole meal amaranth in short dough wheat cookies and noticed that, even at substitution levels of 25%, cookie dimensions and texture were substantially better than those of the wheat control. Incorporation of amaranth or quinoa flour in oat (Inglett and others 2015) or wheat cookies (Chauhan and others 2016) increased dough elasticity. Higher substitution levels of amaranth whole meal increased cookie diameter and spread ratio, but decreased cookie hardness (Chauhan and others 2016). More has been published on the production of cookies containing buckwheat. Addition of increasing levels of buckwheat flour to a wheat flour recipe resulted in inferior properties of short dough cookies (Baljeet and others 2010; Jan and others 2015) and ginger nut biscuits (Filipˇcev and others 2011).

Substitution of increasing levels of dehulled buckwheat flour in a gluten-free cookie formulation based on rice flour provided more viscous and less extensible dough. Furthermore, the overall cookie quality decreases when the buckwheat flour substitution level was raised from 10 to 30% (Hadnaev and others 2013). The incorporation of buckwheat flour in gluten-free sugar-snap cookies resulted in cookies with an oven spread comparable to that of sugar-snap cookies produced from maize flour or whole-meal teff (Mancebo and others 2015).

Strategies to improve the functionality of pseudocereal proteins in dough systems

Enzymes. Transglutaminase (EC 2.3.2.13), a glutamine-γ-glutamyl transferase, catalyzes the formation of iso-peptide bonds between ε-amino groups of lysine residues and the γ-carboxyamide functional group of glutamine residues (Reimikainen and others 2003). Renzetti and others (2008a,b) prove that a transglutaminase promotes covalent cross-links between common buckwheat proteins. In addition, confocal laser scanning micrographs showed that the application of transglutaminase improves the homogeneity of the protein network. This results in more elastic buckwheat dough (Han and others 2013a) and in buckwheat bread of improved crumb softness and elasticity (Renzetti and others 2008b). Transglutaminase treatment triggers the formation of high–MM polymers in all Osborne protein fractions (Renzetti and others 2008a). SE-HPLC chromatograms confirmed that high MM protein structures are formed in the common buckwheat albumin and globulin fraction when treated with transglutaminase, but not in the prolamin and glutelin fractions. The positive effect of transglutaminase treatment on network formation between buckwheat proteins most likely results from the high levels of accessible lysine and glutamine residues.

In the presence of molecular oxygen, glucose oxidase (EC 1.1.3.4) acts as a catalyst for the oxidation of β-D-glucose to hydrogen peroxide and D-glucic acid. The hydrogen peroxide, an oxidizing agent, interacts with the free SH-groups of cysteine residues to give rise to the formation of SS-bonds (Joye and others 2009). Only 1 study deals with the effect of a glucose oxidase treatment on the formation of cross-links between proteins from pseudocereal flour (Renzetti and Arendt 2009). The addition of glucose oxidase did not significantly affect buckwheat batter viscosity or buckwheat bread specific volume and crumb texture. This is likely due to the low availability of free SH-groups in the major globulin fraction, as their subunits are intramolecularly linked via an SS–bond (Table 2) (Renzetti and Arendt 2009; Taylor and others 2016).

Peptidases (EC 3.4.), act by hydrolyzing the peptide bonds linking amino acids in proteins. Treatment with a commercial protease lowered buckwheat batter viscosity as a result of protein hydrolysis, but significantly increased buckwheat loaf specific volume (Renzetti and Arendt 2009). In addition, the center of the crumb showed a very large void.

High-pressure treatment. In general, high-pressure treatment reduces the large number of noncovalent bonds, resulting in (partial) unfolding of the protein. This way, the SH-groups of cysteine residues become more accessible to SH-interchange reactions (Vallons and others 2011). Hence, high levels of free SH-groups are a prerequisite to induce structure formation between proteins by high pressure, which is not the case for buckwheat flour proteins. Consequently, the impact of high-pressure treatment on these proteins is rather limited (Vallons and others 2011). This observation is in agreement with the negative effect of glucose oxidase (see subsection “Enzymes”) on the formation of covalent bonds between buckwheat flour proteins.

Conclusions and Perspectives

The well-balanced amino acid composition and the high levels of dietary fiber, antioxidants, vitamins, and minerals make pseudocereals interesting raw materials for highly nutritious cereal-based food systems. An asset is that people with celiac disease can consume pseudocereals. While prolamins and glutelins are the most abundant protein fractions of true cereals (such as wheat), the proteins of pseudocereals are predominantly albumins and globulins. Although it is general practice to classify cereal grain proteins by using an Osborne fractionation scheme, such scheme is not always rigorously followed for pseudocereals. Especially pseudocereal globulins and glutelins are in most instances not extracted in a standard Osborne protocol. Furthermore, research outcomes on structural properties of pseudocereal proteins are often contradictory. Here, the application of mass spectrometry for characterizing pseudocereal protein sequences (Qian and others 2008; Gao and others 2010; Lagrain and others 2013) would be helpful. Pseudocereals are classified as nonmodel plant species since their genome sequence is currently not available. Hence, only scarce protein sequences are available in online databases. A more thorough understanding of the primary, secondary, and tertiary structures of...
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pseudocereal proteins is required to establish in-depth structure–function relationships.

As demonstrated in this review paper, the basic foaming, emulsifying, and gelling properties of amaranth, buckwheat, and quinoa proteins have been investigated. These techno-functional properties are often similar to or even better than those of industrially important protein sources such as soy protein and casein, which makes them a promising alternative in many food applications. In most cases, in-depth studies on, among others, the kinetics of protein adsorption to the interface and properties of the film at the interface, are missing. Moreover, the majority of the studies on pseudocereal protein functionality cited in this paper focus on protein isolates obtained by alkaline extraction and subsequent IEP at acidic pH. The extraction is, in fact, a pretreatment that can induce changes in protein conformation and/or structure and in some cases can cause (partial) denaturation and thereby affect techno-functional properties, as already demonstrated for foaming and gelling properties. Hence, the outcomes of such studies are not necessarily predictive for the properties of proteins in pseudocereal flour.

Strategies to affect the functionality of pseudocereal flour proteins and, concomitantly, to improve the quality of the end products include the use of enzymes (such as transglutaminases, glucose oxidases, and peptidases), high-pressure treatments, and sourdough fermentation. The most appropriate strategy depends on the raw material used and the end product considered. For instance, the native structure of the major buckwheat globulins seems responsible for the only limited effect of glucose oxidase and high-pressure treatments. In contrast, transglutaminase mediates the formation of cross-links between buckwheat flour albumins and globulins, likely due to high levels of accessible lysine and glutamine residues in the soluble protein fraction. Hence, among the strategies discussed in section “Wheat and gluten-free bread,” transglutaminases appear to have the largest potential to improve the rheological behavior of pseudocereal dough systems.

In conclusion, only very few studies exist on the properties of native pseudocereal proteins, and only limited fundamental studies on pseudocereal proteins (in cereal-based end products) are available. As a result, information on their functionality in establishing end product characteristics is scarce. Food products often consist of foam, an emulsion, a gel, or a combination thereof. Hence, elucidation of the denaturation and aggregation behavior, and of the techno-functional properties of amaranth, buckwheat, and quinoa proteins, can form a basis for improving overall product quality. In addition, the techno-functional properties of both animal and vegetable proteins can be improved by enzyme treatments (Gaspar and de Géees–Favoni 2015; Wouters and others 2016), mild heating, or phosphorylation (Du and others 2002; Li and others 2004; Hayashi and others 2009). Whether, or to what extent, similar techniques can further improve the techno-functional properties of pseudocereal proteins remains largely unknown.

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