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Review

# Amaranth, quinoa and chia protein isolates: Physicochemical and structural properties

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ABSTRACT

An increasing use of vegetable protein is required to support the production of protein-rich foods which can replace animal proteins in the human diet. Amaranth, chia and quinoa seeds contain proteins which have biological and functional properties that provide nutritional benefits due to their reasonably well-balanced aminoacid content. This review analyses these vegetable proteins and focuses on recent research on protein classification and isolation as well as structural characterization by means of fluorescence spectroscopy, surface hydrophobicity and differential scanning calorimetry. Isolation procedures have a profound influence on the structural properties of the proteins and, therefore, on their *in vitro* digestibility. The present article provides a comprehensive overview of the properties and characterization of these proteins.



**Contents**



## 1. Introduction

In addition to their role as a macronutrient, proteins play a key role in food structure through processes such as emulsification, foaming, gelation and dough formation. Food protein supply is presently scarce, and this situation will worsen if the world population continues to increase. As more food protein sources will be needed, research has been focusing on new alternative protein sources [1]. Thus, proteins from seeds, grains, legumes, fish, microbes, algae, and leaves are presently being evaluated [2–6].

An increasing use of vegetable protein is required to support the production of protein-rich foods which can replace animal proteins in the human diet. Otherwise, from a nutritional standpoint, plant proteins can supply sufficient amounts of essential aminoacids for human health requirements [6].

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Soy is an example of how scientific research can add value and diversify the use of vegetable proteins in a wide variety of food products. While soy is the most common alternative protein source to replace animal-based protein, new food products containing proteins from other sources, such as grains, legumes and vegetables, are currently being evaluated [7].

Amaranth (*Amaranthum*), quinoa (*Chenopodium quinoa Willd*) and chia (*Salvia hispanica* L.) are non conventional sources of proteins that have been studied in recent years. They are referred to as pseudocereals, as their seeds resemble in composition and function those of true cereals. In addition, the aminoacid composition of pseudocereal proteins is well balanced, with a high content of essential aminoacids, and high bioavailability. Moreover, pseudocereals are gluten-free products, which represent a significant advance towards ensuring an adequate intake of nutrients in subjects with celiac disease [8].

Amaranth, chia and quinoa have been cultivated from tropical to subtropical regions and were important food crops to Aztec, Mayan and Incan civilizations [8,[9]]. However, their production and use declined significantly after the Spanish conquest. Today, these ancient crops are grown commercially in Mexico, Bolivia, Argentina, Ecuador, Guatemala and Peru [9].

The present review provides a comparative study on some aspects of amaranth, quinoa, and chia proteins based on recent research. This review compares these proteins and focuses on recent research reporting studies on: protein classification and isolation; structural characterization by means of fluorescence spectroscopy, surface hydrophobicity and differential scanning calorimetry; and *in vitro* protein digestibility.

## 2. Seed protein classification and characterization

Seed proteins can be classified on the bases of different criteria such as function and differential solvent solubility, among others.

A classification mainly based on protein function divides seed proteins into three groups: “storage”, “structural and metabolic” and “protective” proteins [10].

Storage proteins are those proteins which are laid down at one stage of the development for future use to supply intermediary nitrogen compounds for biosynthesis at a metabolic active stage [11,12].

Simple proteins were first classified by Osborne [13] based on the differential solubility of each fraction in both aqueous and non-aqueous solvents. This is the most widely used classification for plant proteins. The albumin fraction is obtained from a suspension in water while the globulin fraction is soluble in diluted salt solutions. Prolamins are the alcohol-soluble fraction and glutelins are the most difficult fraction to solubilize, being usually extractable with weak alkalis and acids or dilute detergent solutions [14].

The fractionation and characterization of the different groups of storage proteins from amaranth, quinoa and chia have been reported. Table 1 shows the protein fraction content of some varieties of amaranth, quinoa and chia.

The data presented in Table 1 intend to show not only the proportion of each seed protein fraction but also the evident variability found in this type of data. The solvents used in different works were not the same. In fact, salt concentrations, type of alcohol and the denaturant agents used varied, causing differences in the amount of protein solubilized in each case. Finally, various methods for protein quantification were used, and the reference method of Kjeldahl was not always the method of choice [18]. Colorimetric assays, as bicinchoninic acid, [15] or electrophoresis [16,17] were also applied to protein determination.

Sequential extraction and characterization of amaranth proteins has extensively been performed and has been revised in detail [15,20] and therefore will not be reviewed here.

Quinoa protein fractions have also been characterized in the past century [21]. Albumin fraction has been obtained from dispersion in water, while globulins have been extracted using 0.5 M NaCl. The extraction of the prolamin fraction has been the result of the suspension in a solution containing 95% of ethanol and 0.6% of β-2-mercaptoethanol. A denaturing-reducing buffer (0.0625 M Tris pH 8.1 containing SDS 2% and β-mercaptoethanol 5%) has been used to extract the glutelin fraction. These authors have found that most of the proteins in quinoa seeds can be classified into albumins or globulins. The electrophoretic profile for the albumin fraction shows both 21 definite bands and also smeared bands corresponding to molecular weight below 20 kDa. The globulin fraction contains 8 polypeptides that were visualized in SDS-PAGE: three of them correspond to molecular weights near 36 kDa, two of them to molecular weight values near 29 kDa and three to values of around 25 kDa. The electrophoresis of the prolamin fraction showed no bands and that of the glutelin fraction showed bands that correspond to other bands in albumin or globulin fractions, meaning that some insolubilization degree of these polypeptides might be caused during the protein extraction process. However, Mäkinen et al. [22] have reported that the globulin fraction, also analyzed by means of SDS-PAGE, is composed by ten polypeptides, with molecular weights ranging from less than 20 kDa up to 50 kDa. The electrophoretic pattern shown by these two groups of authors is different, probably due not only to the different electrophoretic protocol, but also to the use of different protein sources and extraction conditions.

Recently, a fractionation procedure to characterize the protein groups from chia defatted flour based on their solubility differences has been performed by Sandoval-Oliveros and Paredes-Loípez [18]. As for the other vegetable proteins, the albumin fraction has been obtained from a suspension of chia flour in water. The pellet was resuspended in 0.5 M NaCl, in order to obtain the globulin fraction. The prolamin fraction was the result of the pellet resuspension in a 70% aqueous isopropanol solution. The resulting pellet was resuspended in a 0.1 M $Na_2B_4O_7.10H_2O$ solution (pH 10), to separate the glutelin fraction. They carried out SDS-PAGE for these samples and found that globulins are the major fraction, as shown in Table 1. The SDS-PAGE pattern shows that there are 11 bands corresponding to albumins and 19 corresponding to globulins, coinciding with five bands that correspond to polypeptides with molecular weights ranging from 50 to 200 kDa. In fact, some authors consider that it is not possible to assure the absence of some globulins in the albumin fraction; thus, they recommend considering these two groups together as one globulin + albumin fraction, for the sake of comparison [15]. The bands corresponding either to the glutelin (4 bands from 20 to 30 kDa) or prolamin (2 bands around 30 kDa) fractions visualized in the SDS-PAGE correspond to bands observed in the globulin fraction. According to Fukushima [23], the glutelin fraction may be classified either as a globulin or glutelin fraction, since he attributes the difficulty in the solubilization of these proteins to the denaturation caused by the processing of the samples (effect of solvents, temperature, among others).

On the other hand, Olivos-Lugo et al. [19] have reported a significantly different proportion of fractions in Mexican chia seeds, with prolamins and glutelins being the most abundant fractions. They found not only different proportions of each fraction, but also a 12.3% of completely insoluble protein. In our opinion, these differences in solubility could be attributed to the different methods applied to obtain the defatted flour, as pointed out above [23].

Table 2 shows the composition of aminoacids of amaranth, quinoa and chia seeds and their protein fraction

Amaranth albumin and globulin are relatively rich in essential Lys aminoacid and sulfur aminoacids, while glutelins are a source of Phe + Tyr and Leu [15]. Barba de la Rosa et al. have reported a higher content of Val in the albumin and globulin fraction [16].