Available online at www.sciencedirect.com
SCIENCE DIRECT
Food Chemistry 100 (2007) 1350–1355
Food Chemistry
www.elsevier.com/locate/foodchem

The content of proteic and nonproteic (free and protein-bound) tryptophan in quinoa and cereal flours

Stefano Comai [a], Antonella Bertazzo [a], Lucia Bailoni [b], Mirella Zancato [a], Carlo V.L. Costa [a], Graziella Allegri [a,*]

[a] Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Padova, via Marzolo 5, I-35131 Padova, Italy
[b] Department of Animal Science, Faculty of Veterinary Medicine, University of Padova, Agripolis-viale dell'Università 16, I-35020 Legnaro (PD), Italy

Received 22 September 2005; accepted 28 October 2005

Abstract

The content of proteic and nonproteic (free and protein-bound) tryptophan and of proteins in quinoa, wheat, rice, maize, barley, oat, rye, spelt, sorghum and millet flours was determined. Protein content and proteic tryptophan of quinoa were similar to that of wheat and spelt, but higher than in other cereals. Free tryptophan in quinoa flour showed values similar to those of wheat, oat and sorghum Kalblank, lower than those of barley, spelt and pearl millet, but higher than in rice, maize, rye, sorghum DK 34 – Alabama hybrid. In addition, nonproteic tryptophan appears bound both to water soluble proteins and to proteins soluble at pH 8.9. The results are discussed regarding the importance of the nonprotein tryptophan fraction, the only one able to enter the brain, that is more easily absorbed, so guarantees a greater amount available for uptake by the central nervous system.

Keywords: Quinoa; Cereals; Proteic tryptophan; Free tryptophan; Protein-bound tryptophan

1. Introduction

Quinoa (*Chenopodium quinoa* Willd. of the Chenopodiaceae family) is a dicotyledonous indigenous plant of the Andes region above 4000 m of altitude. It is considered an excellent pseudocereal for its nutritional characteristics. It is a pseudocereal still widely cultivated in South America (Mujica, 1994), such as Perú, Bolivia, Columbia, Ecuador, Chile and Argentina. Commercial quinoa production in the United States has been successful (Johnson & Croissant, 1989), targeting the grain at the health food sector (Galwey, Leakey, Price, & Fenwick, 1990). Quinoa has received considerable attention as an alternative crop throughout the world, primarily because of its high nutritional value. Agronomists are investigating how to grow Quinoa in suitable areas of the USA and Europe as a new food resource (Galwey et al., 1990; National Research Council, Washington, 1989) because of its high protein content having, in particular, the amino acid composition of seed protein close to the ideal protein balance recommended by the FAO (Gross et al., 1992; Mahoney, Lopez, & Hendricks, 1975) and similar to that of milk (Kozioł, 1992). Quinoa also has a relatively high quantity of vitamins and minerals (Risi & Galwey, 1984) and quinoa seed lipids appear to be a high quality edible vegetable oil, similar in fatty-acid composition to soybean oil (Wood, Lawson, Fairbanks, Robison, & Andersen, 1993). Due to its great food potential, it is being introduced in many other countries. Quinoa is also considered as a potential crop for the National Aeronautics and Space Administration (NASA's Controlled Ecological life Support System – CELSS) (Schlick & Bubenheim, 1993, 1996).

Quinoa is rich in protein (14–16%) (Kozioł, 1990, 1992). On a dry matter basis, Quinoa shows a protein content higher than that found in cereals. The protein is of an exceptionally high quality and is particularly rich in

* Corresponding author. Tel.: +39 049 8275355; fax: +39 049 8275366.
E-mail address: graziella.allegri@unipd.it (G. Allegri).

0308-8146/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2005.10.072

histidine and lysine. Preliminary studies of protein fractionation have shown that the main proteins are albumin, globulin (called Chenopodin) and in a low percentage prolamin (Ballón, De Gomez, & Cuesta, 1982; Kozioł, 1992). Their proportions vary for different species (Prakash & Pal, 1998). The digestibility of quinoa protein was found to be comparable to that of other high quality food proteins (De Simone, Dini, Pizza, Saturnino, & Schettino, 1990). Starch accounts for 52–60% of grain weight. The seeds are used boiled like rice or used to thicken soup or as porridge. Quinoa flour was made into noodles. Its use is, however, complicated due to the bitter taste of seeds because of their saponin content (De Simone et al., 1990; Dini, Schettino, Simioli, & Dini, 2001; Dini, Tenore, Schettino, & Dini, 2001; Mizui, Kasai, Ohtani, & Tanaka, 1990) which forms a soapy solution in water. Saponins are located in the coat of the quinoa seeds and to remove the seed coat it is sufficient to gently grind with a mortar and pestle. However, it was found that saponins do not exert any negative effect on the nutritive quality of the protein (Ruales & Nair, 1992). Some varieties exist in which saponin content is very low or absent (Kozioł, 1990, 1992) as in quinoa harvested in southern Bolivia (S. Juan Altoplano).

Although much has been done on the amino acid composition, there is only very limited information on the free and protein-bound tryptophan contents in quinoa and in cereals.

The purpose of this research was to determine this nonproteic tryptophan in the flour of a Bolivian quinoa sample in comparison to the flours of common cereals.

2. Materials and methods

2.1. Samples

The seeds of quinoa (*Chenopodium quinoa* Willd., variety of *Sajama*), harvested in rural areas of S. Juan (Bolivia) at 4500 m altitude during the year 2003, were used. In addition, the following grain samples of cereals, purchased from the market, were analysed: wheat (*Triticum aestivum*), rice (*Oryza sativa*), maize (*Zea mays*), barley (*Hordeum vulgare*), oat (*Avena sativa*), rye (*Secale cereale*), spelt (*Triticum spelta*), pearl millet (*Panicum miliaceum*), two hybrids of *Sorghum bicolor* (Kalblank and DK 34 – Alabama), Indian sorghum and Indian millet. These two last samples were purchased in a tribal village of Orissa, India in the year 2004.

Seed samples were ground to a fine powder in a mortar with a pestle. The powder was passed through a 300 μm sieve.

2.2. Nonprotein tryptophan extraction

The method was optimized as follows: 1 or 2 g of the dry flour was defatted by suspension in 10 ml of acetone and stirred for 30 min at 37 °C. The sample was centrifuged at 12,000 rpm for 10 min by IEC-Centra-SR centrifuge at 0 °C and the organic layer was removed. An additional 10 ml of acetone was added to the remaining pellet and the sample was shaken for 10 min and centrifuged. The organic layer was removed. The dried sediment was extracted with 10 ml of distilled water for 30 min at 37 °C under shaking; then it was centrifuged and the supernatant, containing the free tryptophan and the water soluble protein fraction, was collected. The residue was re-extracted with a further 10 ml of distilled water for 30 min and, after centrifugation, the supernatants were combined. Extending the extraction time beyond 1 h did not increase the product yield further. An aliquot (5 ml) was ultrafiltered by an Amicon model 12 ultrafiltration cell with an XM-50 Diaflo membrane (Amicon, Oosterhout, Holland) collecting the first 500 μl of ultrafiltrate used to determine the free fraction of tryptophan according to the method of Costa, Bettero, and Allegri (1987). The remaining part of the supernatant was analyzed for the determination of total water soluble nonprotein tryptophan.

The sediment obtained after extraction with water was resuspended in 5 ml of 0.1 M potassium phosphate buffer, pH 8.9, shaken for 30 min and then centrifuged. The supernatant was analysed by HPLC to determine the nonprotein tryptophan eventually bound to water insoluble proteins.

All the samples of cereals were prepared in the same way.

2.3. Analysis of nonprotein tryptophan

The analysis of free form and total nonprotein (free + protein-bound) tryptophan was carried out on a combined HPLC-fluorescence system according to the method of Costa et al. (1987).

2.4. Protein content

Nitrogen was assayed in quinoa and cereal flours (dry matter) by the micro-Kjeldahl method and the nitrogen percentage was converted to crude protein by multiplying by 6.25.

2.5. Analysis of proteic tryptophan

The determination of tryptophan was done in triplicate by HPLC by using the method of Slump, Flissebaalje, and Haaksman (1991) based on the alkaline hydrolysis of flours in $Ba(OH)_2$. The alkaline hydrolysis appears to be the most widely used method for the analysis of proteic tryptophan in food. The hydrolysis was performed at 130 °C for 8 h. After cooling at room temperature, the pH was adjusted to 4.5 with concentrated HCl. A solution of 5-methyl-tryptophan (Sigma Chemical Co.) was added as an internal standard. The volume was adjusted with water to 50 ml. The suspension was mixed and filtered. An aliquot of the filtrate was diluted at least threefold with buffer. The mixture was homogenized and filtered and the filtrate was analyzed by HPLC. The liquid chromatographic equipment consisted of an automatic injector (AS3000), a pump

(P400) and a fluorescence detector (FL 3000) (Spectra System, Thermo Finnigan). The column was a Zorbax extended C18 (3 × 250 mm). The eluting solvent was 0.1 Na-acetate (42.5%)/0.1 M acetic acid (42.5%)/methanol (15%).

The mean values are expressed in mg of tryptophan per 100 g of protein or per 100 g of flour (on a dry matter basis).

3. Results

Table 1 reports the values of protein content and proteic tryptophan in flours of quinoa and common cereals. The values are means ± standard error of three separate analyses. The protein content is expressed as g/100 g dry weight and was estimated using 6.25 nitrogen/protein conversion factor on a dry matter basis. As it is shown, the protein content of quinoa (16.4 g/100 g dry wt) is similar to those of wheat and spelt (16.8 and 16.3 g/100 g dry wt, respectively), but is higher when compared with the flours of cereals like rice, maize, barley, oat, rye, pearl millet and sorghum bicolor, Kalblank hybrid (7.94, 8.94, 11.8, 11.3, 10.7, 11.4, and 8.87 g/100 g dry wt, respectively). The lowest protein content was found for millet harvested in a tribal village of Orissa – India in the year 2004 (4.50 g/100 g dry wt).

In sorghum bicolor, DK 34 – Alabama hybrid, the protein content (5.37 g/100 g dry wt) is markedly lower, not only with respect to other cereals, but also to the sorghum bicolor, Kalblank hybrid (8.87 g/100 g dry wt). Instead, sorghum harvested in a tribal village of Orissa in the year 2004 shows a protein content (7.93 g/100 g dry wt) similar to that of sorghum bicolor, Kalblank hybrid.

Proteins differ greatly in terms of their content in tryptophan calculated on a dry matter basis (Table 1). The results for proteic tryptophan are expressed as the mean ± standard error of determinations made on three replicate hydrolyzates. Quinoa flour shows a proteic tryptophan value (187 mg/100 g dry wt) similar to those of wheat and spelt (195 and 191 mg/100 g dry wt, respectively), but much higher than the other cereals (Table 1). The lowest value was obtained for maize flour (44 mg/100 g dry wt), while the two sorghum hybrids show different values, sorghum bicolor Kalblank being richer than sorghum bicolor DK 34 – Alabama (99 and 60 mg/100 g dry wt, respectively). Similarly, the proteic tryptophan in sorghum from Orissa was low (61 mg/100 g dry wt). Also pearl millet (*Panicum miliaceum*) contains more proteic tryptophan than millet from Orissa (110 and 74 mg/100 g dry wt, respectively).

Considering the values of proteic tryptophan as mg/100 g of protein (Table 1), quinoa flour contains greater amounts of tryptophan (1142 mg/100 g protein) compared with maize and rye flours (491 and 823 mg/100 g protein, respectively), but similar amounts compared to those in flours of other cereals (wheat: 1160; rice: 1221; spelt: 1173; barley: 961; sorghum bicolor Kalblank: 1116; sorghum bicolor DK 34 – Alabama: 1123, pearl millet: 970; oat: 977 mg/100 g protein, respectively). Proteins of the millet flour from Orissa appear to be particularly rich in proteic tryptophan (1644 mg/100 g protein). However, this millet is poor in protein.

Free and nonprotein-bound tryptophan contents, present in aqueous extracts of flours, are reported in Table 2. Quinoa flour contains a free tryptophan amount (2.97 mg/100 g flour, dry wt) similar to that of wheat, oat, and sorghum Kalblank (3.51, 3.57, 3.61 mg/100 g flour, respectively), but lower than in barley, spelt, and pearl millet (5.88, 7.79, 4.87 mg/100 g dry wt flour, respectively). However, the content of free tryptophan in quinoa flour is higher than in rice, maize, rye, sorghum DK 34 – Alabama, Orissa sorghum, and Orissa millet (0.30, 0.66, 2.06, 1.66, 0.93, 1.02 mg/100 g flour, respectively).

Table 2 also reports the contents of protein-bound tryptophan in quinoa and cereal flours expressed as mg/100 g

Table 1
Protein content (g/100 g dry wt) and proteic tryptophan (mg/100 g dry wt and g/100 g protein) in flours of Quinoa and common cereals (mean values ± standard error)

Flours	Protein content[a]	Proteic tryptophan[b]	
	g/100 g dry matter	mg/100 g dry wt	mg/100 g protein
Quinoa (*Chenopodium quinoa*)	16.4 ± 1.2	187 ± 6	1142 ± 36
Wheat (*Triticum aestivum*)	16.8 ± 1.3	195 ± 7	1160 ± 42
Rice (*Oryza sativa*)	7.94 ± 0.54	97 ± 2	1221 ± 26
Maize (*Zea mays*)	8.94 ± 0.60	44 ± 2	491 ± 17
Barley (*Hordeum vulgare*)	11.8 ± 0.9	113 ± 5	961 ± 40
Oat (*Avena sativa*)	11.3 ± 0.8	110 ± 5	977 ± 47
Rye (*Secale cereale*)	10.7 ± 0.7	88 ± 2	823 ± 19
Spelt (*Triticum spelta*)	16.3 ± 1.0	191 ± 5	1173 ± 32
Pearl millet (*Panicum miliaceum*)	11.4 ± 0.7	110 ± 4	970 ± 31
Sorghum bicolor (Kalblank)	8.87 ± 0.50	99 ± 3	1116 ± 34
Sorghum bicolor (DK 34 – Alabama)	5.37 ± 0.20	60 ± 2	1123 ± 33
Sorghum (Orissa – India)	7.93 ± 0.40	61 ± 2	769 ± 22
Millet (Orissa – India)	4.50 ± 0.20	74 ± 0.9	1644 ± 18

[a] Values are averages from three separate determinations and were calculated using 6.25 as nitrogen:protein conversion factor.
[b] Determined by HPLC analysis after alkaline hydrolysis of flours.

Table 2
Comparison of the values (means ± SE) of the free and protein-bound tryptophan (Trp) among the flours of Quinoa and common cereals

Flours	Free Trp (mg/100 g flour[a])	Total protein-bound Trp[b], H_2O soluble fraction (mg/100 g flour[a])	Protein-bound Trp, buffer fraction, pH 8.9 (mg/100 g flour[a])
Quinoa (*Chenopodium quinoa*)	2.97 ± 0.24	4.03 ± 0.13	1.50 ± 0.05
Wheat (*Triticum aestivum*)	3.51 ± 0.22	5.10 ± 0.20	1.36 ± 0.09
Rice (*Oryza sativa*)	0.30 ± 0.02	0.34 ± 0.01	0.13 ± 0.01
Maize (*Zea mays*)	0.66 ± 0.04	0.68 ± 0.01	0.33 ± 0.01
Barley (*Hordeum vulgare*)	5.88 ± 0.18	6.03 ± 0.24	1.85 ± 0.06
Oat (*Avena sativa*)	3.57 ± 0.32	4.12 ± 0.10	0.79 ± 0.03
Rye (*Secale cereale*)	2.06 ± 0.20	3.65 ± 0.35	0.50 ± 0.04
Spelt (*Triticum spelta*)	7.79 ± 0.52	19.33 ± 0.36	2.29 ± 0.20
Pearl millet (*Panicum miliaceum*)	4.87 ± 0.29	4.95 ± 0.21	1.51 ± 0.11
Sorghum bicolor (Kalblank)	3.61 ± 0.25	3.63 ± 0.14	1.52 ± 0.04
Sorghum bicolor (DK 34 – Alabama)	1.66 ± 0.10	2.08 ± 0.08	0.74 ± 0.02
Sorghum (Orissa – India)	0.93 ± 0.03	1.01 ± 0.03	0.29 ± 0.01
Millet (Orissa – India)	1.02 ± 0.03	1.09 ± 0.04	0.42 ± 0.03

[a] On dry matter.
[b] Free + protein-bound Trp (water soluble fraction).

dry wt flour ± SE. As it appears from Table 2, protein-bound tryptophan is present both in the water soluble fraction and in the fraction obtained by extraction at pH 8.9.

With regard to the water soluble fraction, protein-bound tryptophan content in quinoa (4.03 mg/100 g flour) is similar to that in oat, rye, and sorghum Kalblank (4.12, 3.65, 3.63 mg/100 g dry wt flour, respectively), lower than in wheat, barley and pearl millet (5.10, 6.03, 4.95 mg/100 g dry wt flour, respectively), and much lower than in spelt (19.3 mg/100 g flour). In contrast, rice, maize, sorghum Alabama, Orissa sorghum and Orissa millet flours show the lowest values (Table 2).

Tryptophan is also bound to proteins extracted at pH 8.9. In comparison with cereals, quinoa presents a content of protein-bound tryptophan (pH 8.9 fraction) similar (1.50 mg/100 g flour) to that of wheat, pearl millet, and sorghum Kalblank (1.36, 1.51, 1.52 mg/100 g flour, respectively), lower than spelt and barley (2.29 and 1.85 mg/100 flour, respectively), but higher than rice, maize, oat, rye, sorghum Alabama, Orissa sorghum, and Orissa millet (0.13, 0.33, 0.79, 0.50, 0.74, 0.29, 0.42 mg/100 g flour, respectively).

4. Discussion

Tryptophan is an essential amino acid in mammals. It is an indispensable precursor for biologically important compounds, such as the neurotransmitter serotonin and the hormone melatonin, and it is involved in the regulation of several biological processes (Musajo & Benassi, 1964; Wolf, 1974). It is required for protein synthesis and is the major source of body stores of the nicotinamide-containing coenzymes NAD and NADP (Bender & McCreanor, 1982) involved in almost all biogenetic and biosynthetic pathways of the body.

Tryptophan is the only amino acid that in man is bound to circulating plasma albumin in amounts ranging between 80% and 90% (McMenany & Oncley, 1958). The small free fraction (20–10%), the only one able to enter the brain, has great functional importance, since its availability appears to be vital in controlling synthesis of serotonin (Gessa & Tagliamonte, 1974; Knott & Curzon, 1972; Tagliamonte, Biggio, Vargiu, & Gessa, 1973).

It is known that tryptophan is the less represented amino acid in the proteins of cereals which, together with legumes, constitute the main sources of protein used for human food. Currently, nutritionists are evaluating quinoa as an alternative to cereals in the human diet for its nutritional value, being that its nutritional profile is better than that of common cereals (Kozioł, 1992; Schlick & Bubenheim, 1993) and makes quinoa a crop of considerable value and an ideal candidate crop for NASA's Controlled Ecological Life Support System (Schlick & Bubenheim, 1993), meeting the needs of humans on long-term space missions. One of the problems in connection with utilizing of quinoa in human diets is its bitter taste due to the presence of saponins (De Simone et al., 1990; Dini et al., 2001; Dini, Tenore et al., 2001; Mizui et al., 1990). However, it was found that quinoa cultivated in the area of Sajama (S. Juan Altoplano, Bolivia) is free of saponins, as also reported by Schlick and Bubenheim (1993) and can be processed directly for food (Food & Agriculture Organization of the United Nations, Rome, 1989).

Our results show that, on a dry matter basis, quinoa flour contains more protein compared with rice, maize, barley, oat, rye, millet, sorghum, and similar content to that of wheat and spelt, and that the value is similar to that reported in the literature (Kozioł, 1992). Our protein contents in rice and barley are similar but, for wheat and maize they are different in respect to those reported by Duke and Atchley (1986).

As regards the analysis of proteic tryptophan, we chose alkaline hydrolysis that appears to be the most widely used method in food and foodstuffs (Friedman & Cuq, 1988). Proteic tryptophan, too, is higher in quinoa than in the common cereals analyzed (Table 1) with the exception of

wheat and spelt, which show similar contents. The lowest content of proteic tryptophan has been found in maize, in which tryptophan is a nutritionally second-limiting amino acid after lysine (Benevenga & Cieslak, 1978; Bozzini & Silano, 1978; Hassen et al., 1986).

Regarding nonprotein tryptophan, our data indicate that quinoa and cereals contain both free and protein-bound tryptophan. Free tryptophan content in quinoa is quite similar to that of wheat, oat, sorghum (Kalblank) and higher than that of rice, maize, rye, sorghum (DK 34 – Alabama). However, the values of free tryptophan in quinoa are much lower than those of barley, spelt, and pearl millet. Rice and also maize showed to have the lowest content of free tryptophan. In addition, pearl millet contains much more free tryptophan than millet from Orissa and sorghum bicolor (Kalblank) much more than sorghum DK 34 – Alabama and sorghum from Orissa.

Protein-bound tryptophan is present in quinoa and, in all cereals analyzed, both linked to the protein water soluble fraction and to the protein fraction extractable at pH 8.9. Spelt flour shows the highest levels of tryptophan linked to both water soluble and buffered protein fractions, with respect to quinoa and other cereals. Barley, wheat, pearl millet, oat, quinoa, and sorghum (Kalblank) present intermediate values, whereas rice contains the lowest amount of protein-bound tryptophan of the other crops considered.

Very little tryptophan is linked to proteins in the fraction at pH 8.9. Spelt shows higher concentrations with respect to quinoa and other cereals, whereas the lowest content has been observed in rice.

Comparing the levels of nonprotein and proteic tryptophan among the crops analyzed, it appears that they are higher in quinoa, wheat and particularly in spelt than in most other cereals.

The nutritional value of a food is determined by its relationship with protein quality which depends first of all on its amino acid content, digestibility, influence of anti-nutritional factors, and the ratio of tryptophan to large neutral amino acids. Quinoa appears to have not only a high protein content, but also a desirable amino acid composition, as reported in the literature, and a high concentration of tryptophan, usually the second most deficient amino acid in cereals, which are an essential part of daily nutrition.

In addition, the high content of nonprotein tryptophan, more easily absorbable, could have the effect of increasing the availability of this amino acid to the brain, thus influencing the synthesis of the neurotransmitter serotonin.

Our previous studies (Zanardo, Stocchero, Biasiolo, Costa, & Allegri, 1987) indicated that serum levels of total tryptophan in newborn babies fed at the breast or with bovine milk and soybean formulas were similar on days 2 and 6 after birth, in spite of the higher content of this amino acid in human milk. However, the levels of the serum free form of tryptophan were significantly higher in breast-feeding infants than those obtained in infants fed with bovine milk formula.

In fact, colostrum contains much more nonprotein tryptophan than mature human and bovine milk and soybean-formula (Allegri, Biasiolo, Costa, Bettero, & Bertazzo, 1993). Therefore, nonprotein tryptophan could guarantee a greater amount available for uptake by the central nervous system.

Acknowledgment

This work was supported by grants from the Ministero dell'Università e della Ricerca (Cofin. 2004), Rome (Italy).

References

Allegri, G., Biasiolo, M., Costa, C., Bettero, A., & Bertazzo, A. (1993). Content of non-protein tryptophan in human milk, bovine milk and milk- and soy-based formulas. *Food Chemistry, 47*, 23–27.

Ballón, E., De Gomez, V. M., & Cuesta, A. (1982). Estudio de proteinas en variedades de quinua. In *Congreso Internacional de Cultivos Andinos*. 8–12 Febrero 1982. La Paz, Bolivia (pp. 29–34). La Paz, Bolivia Ministerio de Asuntos Campesinos y Agropecuarios.

Bender, D. A., & McCreanor, G. M. (1982). The preferred route of kynurenine metabolism in the rat. *Biochimica Et Biophysica Acta, 717*, 56–60.

Benevenga, N. J., & Cieslak, D. G. (1978). Some thoughts on amino acid supplementation of proteins in relation to improvement of protein nutriture. *Advances in Experimental Medicine and Biology, 105*, 379–400.

Bozzini, A., & Silano, V. (1978). Control through breeding methods of factors affecting nutritional quality of cereals and grain legumes. *Advances in Experimental Medicine and Biology, 105*, 249–274.

Costa, C., Bettero, A., & Allegri, G. (1987). Rapid measurement of tryptophan, 5-hydroxytryptophan, serotonin and 5-hydroxyindoleacetic acid in human CSF by selective fluorescence and high pressure liquid chromatography. *Giornale Italiano Chimica Clinica, 12*, 307–312.

De Simone, F., Dini, A., Pizza, C., Saturnino, P., & Schettino, O. (1990). Two flavonol glycosides from Chenopodium quinoa. *Phytochemistry, 29*, 3690–3692.

Dini, I., Schettino, O., Simioli, T., & Dini, A. (2001). Studies on the constituents of Chenopodium quinoa seeds: isolation and characterization of new triterpene saponins. *Journal of Agricultural and Food Chemistry, 49*, 741–746.

Dini, I., Tenore, G. C., Schettino, O., & Dini, A. (2001). New oleanane saponins in Chenopodium quinoa. *Journal of Agricultural and Food Chemistry, 49*, 3976–3981.

Duke, J. A., & Atchley, A. A. (1986). *CRC handbook of proximate analysis of higher plant*. Boca Raton, FL: CRC Press.

Food and Agriculture Organization of the United Nations, Rome. (1989). In *Utilization of tropical foods: Cereals* (pp. 1–114). Food and Nutrition Paper 47/1.

Friedman, M., & Cuq, J. L. (1988). Chemistry analysis, nutritional value and toxicology of tryptophan in food. A review. *Journal of Agricultural and Food Chemistry, 36*, 1079–1093.

Galwey, N. W., Leakey, C. L. A., Price, K. R., & Fenwick, G. R. (1990). Chemical composition and nutritional characteristics of quinoa (Chenopodium quinoa Willd). *Food Sciences and Nutrition, 42*, 245–261.

Gessa, G. L., & Tagliamonte, A. (1974). Possible role of free serum tryptophan in the control of brain tryptophan level and serotonin synthesis. *Advances in Biochemical Psychopharmacology, 11*, 119–131.

Gross, R., Koch, F., Malaga, I., De Miranda, A. F., Schoenberger, H., & Trugo, L. C. (1992). Chemical composition and protein quality of some local Andean food sources. *Food Chemistry, 34*, 25–34.

Hassen, M. M., Mertz, E., Kirleis, A. W., Ejet, A. G., Axtell, J. D., & Villegas, E. (1986). Tryptophan levels in normal and high-lysine sorghums. *Cereal Chemistry, 63*, 175–176.

Johnson, D. C., & Croissant, R. L. (1989). Quinoa production in Colorado. Serv. Action – Colorado State University. Coop. Ext., 1989, No. 112.

Knott, P. J., & Curzon, G. (1972). Free tryptophan in plasma and brain tryptophan metabolism. *Nature, 239*, 452–453.

Kozioł, M. J. (1990). Afrosimetric estimation of threshold saponin concentration for bitterness in quinoa. *Journal of the Science of Food and Agriculture, 54*, 211–219.

Kozioł, M. J. (1992). Chemical composition and nutritional evaluation of quinoa. *Journal of Food and Computational Analysis, 5*, 35–68.

Mahoney, A. W., Lopez, J. G., & Hendricks, D. G. (1975). An evaluation of the protein quality of quinoa. *Journal of Agriculture and Food Chemistry, 23*, 190–193.

McMenany, R. H., & Oncley, J. L. (1958). The specific binding of L-tryptophan to serum albumin. *Journal of Biological Chemistry, 233*, 1436–1447.

Mizui, F., Kasai, R., Ohtani, K., & Tanaka, O. (1990). Saponins from the bran of Quinoa, Chenopodium Quinoa Willd, II. *Chemical & Pharmaceutical Bulletin, 38*, 375–377.

Mujica, A. (1994). Neglected crops: 1492 from a different perspective. In *FAO plant production and protection*; No. 26; Rome, Italy, FAO.

Musajo, L., & Benassi, C. A. (1964). Aspects of disorders of the kynurenine pathway of tryptophan metabolism in man. *Advances in Clinical Chemistry, 7*, 63–135.

National Research Council. (1989). *Lost crops of the Incas: Little-known plants of the andes with promise for worldwide cultivation* (pp. 149–161). Washington, DC, National Academic Press.

Prakash, D., & Pal, M. (1998). Chenopodium quinoa: changes in amino acid composition in seed during maturity. *International Journal of Food Sciences and Nutrition, 49*, 285–288.

Risi, J., & Galwey, N. W. (1984). The Chenopodium grains of the Andes: Inca crops for modern agriculture. *Advances in Applied Biology, 10*, 145–216.

Ruales, J., & Nair, B. M. (1992). Nutritional quality of the protein in quinoa (Chenopodium quinoa, Willd) seeds. *Plant Foods for Human Nutrition, 42*, 1–11.

Schlick, G., Bubenheim, D. L. (1993). *An emerging "new" crop with potential for CELSS* (pp. 1–6). NASA Technical Paper 3422; Ames Research Center: Moffett Field, CA, National Aeronautics and Space Administration.

Schlick, G., & Bubenheim, D. L. (1996). Quinoa: Candidate crop for NASA's Controlled Ecological Life Support Systems. In J. Janick (Ed.), *Progress in new crops* (pp. 632–644). Arlington, VA: ASHS Press.

Slump, P., Flissebaalje, T. D., & Haaksman, I. K. (1991). Tryptophan in food proteins: a comparison of two hydrolytic procedures. *Journal of the Science of Food and Agriculture, 55*, 493–496.

Tagliamonte, A., Biggio, G., Vargiu, L., & Gessa, G. L. (1973). Free tryptophan in serum controls brain tryptophan level and serotonin synthesis. *Life Sciences, 12*, 277–287.

Wolf, H. (1974). Studies on tryptophan metabolism in man. *Scandinavian Journal of Clinical & Laboratory Investigation, 136*, 1–186.

Wood, S. G., Lawson, L. D., Fairbanks, D. J., Robison, L. R., & Andersen, W. R. (1993). Seed lipid content and fatty acid composition of three quinoa cultivars. *Journal of Food and Computational Analysis, 6*, 41–44.

Zanardo, V., Stocchero, L., Biasiolo, M., Costa, C., & Allegri, G. (1987). Dietary proteins and serum tryptophan levels in full-term newborn infants. In D. A. Bender, M. H. Joseph, W. Kochen, & H. Steinhart (Eds.), *Progress in tryptophan and serotonin research* (pp. 185–188). Berlin, Germany: W. deGruyter.