*Agronomía*

# Phytase activity and comparison of chemical composition, phytic acid P content of four varieties of quinoa grain (*Chenopodium quinoa* Willd.)

## Actividad de la fitasa y comparación en la composición química, contenido de ácido fítico en cuatro variedades de quinua (*Chenopodium quinoa* Willd.)

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## Abstract

Quinoa (*Chenopodium quinoa* Willd.), is a plant that has been cultivated in the Andean region of Bolivia, Peru, Ecuador and Colombia. Its importance is due to the high proteins content and essential amino acids of its grain. The aim of this research was to investigate the relations between phytic acid P content and phytase activities of varieties quinoa Nariño-Colombia (QC), quinoa Anapqui's-Bolivia (QBA), quinoa -IICA 020 Oruro-Bolivia (QB) and quinoa Huancavelica-Peru (QP). We found significant differences in the proportions of protein, fat, fibre, and ash among the four varieties. The analysis of essential amino acids showed that these varieties were rich in arginine, leucine, phenylalanine, lysine and valine, and tyrosine a semi essential amino acids. The fat fraction of all varieties contained high concentration of oleic acid, linoleic acid, α-linolenic acid and palmitic acid. The varieties were rich in phosphorus and calcium. The phytic acid P proportion in total P, in the QC (19,64%) was significantly lower than in other three varieties. In the variety QC (1052 FTU/kg) we found high concentration of phytase. A significant negative correlation (r=-0.89) was found between the concentration of phytase activity and phytic acid P among the four varieties.

**Key words:** Amino acids, Fatty acids, Grain, Phytase, Phytates.

## Resumen

Quinua (*Chenopodium quinoa* Willd.) es una planta que ha sido cultivada en las regiones andinas de Bolivia, Perú, Ecuador y Colombia. Su importancia se debe al alto contenido de proteínas y de aminoácidos esenciales en su grano. El objetivo principal en la investigación fue encontrar la relación entre el ácido fítico y la actividad de la fitasa en las variedades de quinua Nariño procedente de Colombia (QC), quinua Anapquis (QBA) y quinua -IICA 020 Oruro (QB) procedentes de Bolivia, y quinua Huancavelica de Perú (QP). Se encontraron diferencias significativas en las proporciones de proteína, grasa, fibra y ceniza entre las cuatro variedades. El análisis de los aminoácidos esenciales mostró que las variedades de quinua tienen altas concentraciones de arginina, leucina, fenilalanina y lisina, y tirosina como aminoácidos semi-esenciales. La fracción de grasa presentó concentraciones altas de ácido oleico, linoleico, α-linolénico y ácido palmítico. Las variedades presentaron altos contenidos de P y Ca. El ácido fítico en QC (19.64%) fue significativamente más bajo que en las otras variedades. En la variedad

QC (1052 FTU/kg) se encontraron altas proporciones de actividad de la fitasa. Se encontró relación significativa y negativa (r = -0.89) entre la actividad de la fitasa y el ácido fítico en todas las variedades.

**Palabras clave:** Ácidos grasos, amino ácido, fitasa, fitatos, granos, quinua

## Introduction

The quinua crop is important for food security programmes in the Andean region of Latin America, due to the critical situation of crop production for humans. The interest in quinoa is due to its good nutritional value, in particular the high quality of the protein with their essential amino acid composition (FAO, 1989) and fatty acids (Ando *et al.*, 2002), specifically oleic acid (24.8%), linoleic acid (52.3%), and linolenic acid (8.7 %) and mineral content (Ruales and Nair, 1993). Also, the quinoa grain contains some anti-nutritious compounds such as saponins, phytates, tannins, and protease inhibitors (Ruales and Nair, 1993). Phytates form complexes with minerals and are responsible for low utilization of phosphorus (P) and chelation of divalent minerals, which decreases the bioavailability of many essential minerals (Reddy and Sathe, 2002; Reddy *et al.*, 1989; Marounek, 2010). Nevertheless, the occurrence of endogenous enzymes as phytase that plays a key role in the biochemical P-cycling process, have been highly effective in catalyzing phytic acid dephosphorylation (Berry *et al.*, 2007) and release of calcium, iron, zinc, and other metals (Lei and Porres, 2003), due to the fact that previous studies have shown that the cereals have a high enzymatic activity improving the available phytate (Eeckhout and De Paepe, 1994). However, the phytase activity of the quinoa have not been studied previously, for this reason we made an assessment on the phytase activity of the quinoa grain and comparison of the chemical composition, phosphorus, calcium, phytic acid P content of the quinoa grain in four varieties (Nariño variety-Colombia, QC; Anapqui's quinoa, QBA and Commercial-IICA - 020 – Oruro- Bolivia, QB; and Huancavelica-Peru, QP), using as a control containing the wheat phytase. In this study we evaluated the following hypothesis: (1) quinua grain has phytasa activity; (2) the four varieties have significant statistical differences in chemical composition and (3) there is a negative relationship between phytic acid P contents and phytase activity

## Materials and methods

### Quinoa varieties

Four varieties of quinoa were used in this study: quinoa Nariño variety from Colombia (which had a prior desaponification process), quinoa commercial "Anapqui's" and -IICA - 020–Oruro from Bolivia and quinoa Huancavelica from Peru. Following the study of Eeckhout and De Paepe (1994), we used as a control wheat grain, because were a reference value of the phytase activity.

### Chemical analysis

The analysis of chemical composition of quinoa grain was carried out in the Research Institute of Animal Production and the Institute of Animal Physiology and Genetics of the Academy Sciences in Czech Republic. Quinua grain (DM) were analysed crude protein, fat, crude fiber and ash were determined using automatic analyzers (model Kjeltec Auto 1030, Soxtec 1043 and Fibertec 2010 from Tecator AB and SKA-TEC Ltd. Company Prague, CR). Analysis of total P content was determined in grain samples using the vanadate molybdate reagent (OAC, 1980). The phosphorus concentration was measured with a spectrophotometer at $\lambda$ = 820 nm and molybdate reagent (3.4 mM $(NH_4)_6 Mo_7O_{24}$), and 20% (w/v) ascorbic acid. After one hour, the absorbance was measured with a spectrophotometer, which was calibrated with 10 mM $KH_2PO_4$ standard solution. Ca was quantified by atomic absorption spectrometry (Solaar M-6, TJA Solutions, UK). The amino acids content was determined after acid hydrolysis and oxidative hydrolysis by chromatography, with an automatic amino acid analyzer AAA 400-INGOS Prague, CR, equipped with Ostion LG ANB ion-exchange column. Alkaline trans-methylation of extracted FA was carried out according to ISO 5509 (ISO 5509, 2000). Gas chromatography of methyl ester was done with a HP 6890

gas chromatography (Agilent Technologies, Inc.) with a programmed 60m DB-23 capillary column (J&W Scientific, Folsom, USA). Percent of fatty acid were measured on the basis of retention times corresponding to the standards protocol.

**Determination of the phytase activity**

The phytase activity of the grain was determined as described by Eeckhout and De Paepe (1994). The quinoa samples and control (200 mg DM) were weighed into 50 ml volumetric flasks, and then flasks were filled with a solution of Na-phytate (1.722 g of Na-phytate (Sigma P 3168 from rice), 180 ml $H_2O$, and 820 ml of 0.25 M acetate buffer, pH 5.5). The flasks were shaken for 15 min and then placed in a water bath at 37°C. After 10 minutes incubation, a 2 ml portion of incubates was transferred to a test tube containing 2 ml of 10% trichloro-acetic acid (TCA). After another 60 minutes, another 2 ml portion of incubated was transferred to a test tube containing 2 ml of 10% TCA. The contents of both tubes were filtered through a filter paper and 1 ml of the filtrate was transferred to a cuvette, together with 1 ml of a freshly prepared colour reagent. The colour reagent was a mixture of four parts of Solution A (15 g of $(NH_4)_6Mo_7O_{24}.4H_2O$, 55 ml of concentrated sulphuric acid and $H_2O$ to 1L) and one part of Solution B (27 g of $FeSO_4$ $7H_2O$, a few drops of concentrated sulphuric acid and $H_2O$ to 250 ml). Absorbance was measured in a spectrophotometer at 700 nm and compared with a calibration series containing 1 ml of 10% TCA per cuvette, 1 ml of P standard solution (0, 10, 20, 30 µmol P $ml^{-1}$ ) and 2 ml of colour reagent. Phosphorus measured in the 10 minutes incubate was taken as a blank; therefore only the difference in optical density between the 70 min incubate and the 10 min incubate was attributed to phytase activity. The phytase unit described in this protocol is defined as amount of phytase activity which liberates inorganic phosphorus from a 0.0015 M Na-phytate solution, at a rate of 1µmol $min^{-1}$ at pH 5.5 and 37°C.

**Determination of phytic acid**

Phytic acid concentration was estimated by the capillary isotachophoretic method (Dušková *et al*., 2001; Marounek *et al*., 2008, Marounek, 2010). 50 ml of 3.5 % HCl was added to a 5g sample of the varieties and control. After shaking at room temperature (18 °C) for 1h, the mixture was diluted in 100 ml with 3.5% HCl and then centrifuged at 15000 r.p.m for 20 minutes at 4°C. The supernatant was separated from the precipitate and transferred to a volumetric flask (20 ml). The pH of the supernatant (1.5 or 2 ml) was adjusted to 6.0-6.4 with HCl and the volume was brought to 100 ml with deionised water. The solution was analysed by isotachophoresis. The operating conditions were recommended as initial current 70 µA (15µA during detection); leading electrolyte (pH 6.2) containing HCl (10mM), BTP (5.5mM) and HPMC (1g $l^{-1}$); terminating electrolyte (pH 6.2) containing 5mM MES. The time of separation varied from 25 to 35 minutes. An external calibration method was used with solutions of the dodecasodium salt of phytic acid as standards. Seven calibration points were measured in the concentration range from 10 to 120 µM and analysed by isotachophoresis.

**Statistical analysis**

The experiment was developed under a complete randomized statistical design. Multiple one-way analysis of variance (ANOVA) was performed to determine if the chemical compositions varied between varieties. Tukey Kramer *post-hoc* multiple comparison tests were used to determine the significance of results (Sokal and Rohlf, 1995). Four Pearson correlations were used to examine the relations between the concentration of phytic acid and phytase activity (Sokal and Rohlf, 1995). All statistical tests were conducted using Minitab 15 (Minitab, Inc., 2006) software.

## Results and discussion

**Proximal composition of quinoa grain**

The protein content was significantly higher in QC (17.3 %) than other three varieties (Table 1).

Analyses of the quinoa in this study as well as in previous studies have reported similar ranges in quinoa's protein content. Studies by Mendoza (1993) have found that

**Table 1.** Chemical composition of quinoa grain from Colombia, Bolivia and Peru

| Indicator | QC[1] | QBA | QB | QP |
|---|---|---|---|---|
| | (g/100 g DM) | | | |
| Protein* | 17.3**a** | 10.7 **c** | 7.9 **d** | 15.0 **b** |
| Fat | 5.1b | 7.1a | 7.2a | 5.6b |
| Fibre | 6.0c | 6.8a | 6.3b | 5.9d |
| Ash | 3.3a | 2.2d | 2.4c | 2.8b |
| | g/ kg DM | | | |
| Ca | 1.72a | 0.98c | 0.88d | 1.12b |
| P | 4.97c | 4.50a | 4.44d | 5.08b |

[1] QC: *Nariño* variety from Colombia; QBA: Commercial from Bolivia: Anapqui's quinoa; QB: Commercial -*IICA - 020 – Oruro* from Bolivia; QP: *Huancavelica* variety from Peru. *N x 6.25. All values are on a dry basis (%). Means followed by the different letter are significantly different (P<0.05).

Colombia varieties (*San Juan, Puerres,* and *Mocondino*) from Nariño and Cauca region have high percent of protein between 16.86 % and 19.99 %. By other hand, studies in Peruvian quinoa varieties show values near of 12.2 % (Improta and Kellems, 2001; Gonzalez *et al.*, 1989), whereas in Bolivian varieties as *Sajima* show a 16.4% protein content (Comai *et al.*, 2007), being higher that reported in this study. The saponin content may have different effects in protein values, as indicated by studies in the content for sweet (14.8%) and bitter (15.7%) quinoa, showing that protein content is higher in bitter quinoa than in the sweet varieties (Wright *et al.*, 2006). Other factors that can be affects the protein values in the grain are soil fertility and fertilization agricultural practices. For example, in fertilization treatments have found that after an increase of the fertilizer level up to 470 kg N $ha^{-1}$ resulted in higher dry matter, true protein and amino acid contents in quinua (Thanapornpoonpong *et al.*, 2008).

In this study, the fat content in QC (5.1% dry matter) was significantly lower than QB, QBA and QP (Table 1). The fat content in Bolivian quinoa varieties were higher (7.2 %) in comparison with reported in the literature (5.9 % in grain), but the content can differ, because of by manufacturing processes such as dehulling (4.5 %) and physiological seed processes such as germination (7.2-8.8%) (Park and Morita, 2004). Koziol (1993) reported that oil content in the quinoa grain was negatively correlated with protein content (r = -0.910), and this result can be explained by the low protein content in Bolivian quinoa and its high percent fat.

The fibre content (Table 1) was significant higher in QBA (6.8%). In another study reported values of 2.1 % and 3.8 % (Corredor, 2003). In this study, ash content was significant different among the four varieties, being higher in QC (3.3%). The percent ash was similar that reported by others authors 1.2 % and 3.8% (Corredor, 2003; FAO, 1989; Cardozo and Tapia, 1979).

In this study, we found the highest level of phosphorus in QP (5.08 g $kg^{-1}$ DM), with significant variation (P<0.05, Table 1). Similar value was reported in literature for Peruvian varieties (Ruales and Nair, 1993). Other studies have been reported a range value between 1.29 to 3.53 g $kg^{-1}$(Mujica *et al.*, 2001; Chauhan *et al.*, 1992).

The calcium levels showed a significant difference (P<0.05) among varieties with levels from 0.885 to 1.72 g $kg^{-1}$ DM (Table 1). Previous studies found Ca values of 0.874 g $kg^{-1}$ (Ruales and Nair, 1993), 1.10 g $kg^{-1}$ (Chauhan *et al.*, 1992) and 1.14 to 2.28 g $kg^{-1}$ (Mujica *et al.*, 2001). However, these values can be different depending of grain processing. For example, calcium content is reduced with the processes of dehulling (Chauhan *et al.*, 1992).

Amino acid composition of the quinoa grain showed that all varieties were particularly rich in arginine, leucine, phenylalanine, lysine, valine and tyrosine (Table 2). In the essential amino acids composition, the highest percentage was obtained in QB as well as QBA, followed by QP and QC. Although protein content was significantly lower in QBA and QB. This variation in amino acids content may be due to other factors such as the quinoa varieties (Improta and Kellems, 2001), maturity process (Prakash and Pal, 1998), and nitrogen fertilization (Thanapornpoonpong *et al.*, 2008). The high values of arginine, cysteine and tryptophan found in quinua grain have great importance to improve human health, due to these amino acids function as immunonutrients (Suchner *et al.*, 2000).

Linoleic, oleic and α-linolenic acids were more abundant fatty acids in quinoa (Table 3). In the saturated fatty acid group, palmitic acid showed the higher values (9.75% of fat) where as in the unsaturated fatty acid group was oleic acid had the highest concentration (28.337%) in QP. By other hand, in the polyunsaturated group we found a high proportion of linoleic acid in QC (56.44 %) and α-linolenic in QBA and QB (8.7%).

The saturated, unsaturated and polyunsaturated fatty acids concentration found in this study were similar to those reported by other authors (Ruales and Nair, 1993; Ando *et al.*, 2002; Park and Morita, 2004). The unsaturated lipids specifically, polyunsaturated fatty acids (PUFA) have also been reported to influence the immune system (Suchner *et al.*, 2000), cardiovascular disease, mediators of inflammatory response, prevention of atherosclerosis (Bhattacharya *et al.*,2006) and have a positive effect on calcium and bone metabolism (Doyle *et al.*, 2005).

**Table 2.** Nutritional classification of amino acids of quinoa [1]

| Nutrient | QC | QBA | QB | QP |
|---|---|---|---|---|
| **Protein (%)** | 17.3 | 10.7 | 7.9 | 15.0 |
| **Essential amino acids** | | S.D | | |
| Arginine | 14.72 ± 0.286c | 19.49 ± 0.245b | 24.88 ± 0.305a | 13.17 ± 0.099d |
| Histidine | 3.43 ± 0.036d | 5.13 ± 0.066b | 7.29 ± 0.062a | 3.78± 0.043c |
| Isoleucine | 4.67 ±0.012d | 6.86 ± 0.325b | 9.02 ± 0.538a | 4.72 ± 0.151d |
| Leucine | 8.27 ± 0.045c | 12.31 ± 0.424b | 16.51 ± 0.573a | 8.52 ± 0.212c |
| Lysine | 6.90 ± 0.233c | 9.85 ± 0.232b | 12.97 ± 1.01a | 6.94 ± 0.151c |
| Methionine | 1.32 ± 0.053c | 1.94 ± 0.013b | 2.57 ± 0.304a | 1.43 ± 0.056c |
| Phenylalanine | 6.99 ± 0.159c | 10.15 ± 0.471b | 13.19 ± 0.681a | 6.98 ± 0.113c |
| Threonine | 4.57 ± 0.254d | 6.88 ± 0.272b | 9.33 ± 0.188a | 4.88 ± 0.151c |
| Valine | 5.81 ± 0.237c | 8.65 ± 0.312b | 11.40 ± 0.708a | 5.93 ± 0.321c |
| **Semi essential amino acids** | | | | |
| Cysteine | 1.91 ± 0.078d | 2.42 ± 0.059b | 3.61 ± 0.286a | 2.19 ± 0.146c |
| Tyrosine | 5.41 ± 0.184d | 7.97 ± 0.123b | 11.39 ± 0.34a | 5.71 ± 0.203c |
| **Nonessential amino acids** | | | | |
| Asparagine | 11.01 ± 0.147c | 16.61 ± 0.537b | 22.05 ± 0.681a | 11.20 ± 0.473c |
| Serine | 5.17 ± 0.131c | 7.43 ± 0.252b | 10.15 ± 0.313a | 5.21 ± 0.189c |
| Glutamic acid | 15.09 ± 0.430c | 22.12 ± 0.604b | 28.47 ± 0.466a | 14.87 ± 0.264c |
| Proline | 4.07 ± 0.250c | 6.38 ± 0.464b | 8.44 ± 0.26c | 4.27± 0.245c |
| Glycine | 7.54 ± 0.307c | 10.28 ± 0.491b | 13.78 ± 0.600a | 7.26 ± 0.104c |
| Alanine | 6.04 ± 0.094c | 9.06 ± 0.451b | 12.27 ± 0.60a | 6.34 ± 0.066c |

[1] Data are the means and SD for QC: Nariño variety from Colombia; QBA: Commercial from Bolivia: Anapqui's quinoa; QB: Commercial -IICA - 020 – Oruro from Bolivia; QP: Huancavelica variety from Peru. Means followed by the different letter are significantly different (P<0.05).

**Table 3.** Fatty acid composition of fat in quinoa seeds (g/100g *fat*)[1].

| Description | Shorthand designation | QC[1] | QBA | QB | QP |
|---|---|---|---|---|---|
| **Saturated fatty acids** | | | | | |
| palmitic | C16:0 | 9.687a | 9.360b | 9.235b | 9.753a |
| stearic | C18:0 | 0.587c | 0.744b | 0.756b | 0.946a |
| arachidic | C20:0 | 0.434a | 0.459a | 0.465a | 0.544a |
| **Monounsaturated fatty acids** | | | | | |
| oleic | C18:1(n-9) | 20.418d | 26.77c | 27.141b | 28.337a |
| vaccenic (asclepic) | C18:1(n-7) | 1.041c | 1.548b | 1.635a | 1.506b |
| gondoic | C20:1(n-9) | 1.299a | 1.397b | 1.433a | 1.453a |
| **Polyunsaturated fatty acids** | | | | | |
| linoleic | C18:2(n-6) | 56.435a | 48.128b | 47.818c | 46.525d |
| α-linolenic | C18:3(n-3) | 6.721c | 8.814a | 8.670a | 6.968b |
| EPA | C20:5(n-3) | 1.142b | 1.186b | 1.200a | 1.222a |

[1] QC: Nariño variety from Colombia; QBA: Commercial from Bolivia: Anapqui's quinoa; QB: Commercial -IICA - 020 – Oruro from Bolivia; QP: Huancavelica variety from Peru. Means followed by the different letter are significantly different (P<0.05).

**Phytic acid P content and phytase activity of the quinoa grain**

We found a significant differences (P<0.05) of the phytic acid P in total P in the four quinoa varieties and the control (Table 4). The higher concentrations of phytic acid P in grain were obtained in QBA (26.95%), QB (33.6%) and QP (32.17 %). However, the manufacture processes and the germination can decrease the phytic acid content (Cheeke, l995; Kyriakidis *et al.*, 1998; Centeno *et al.*, 2001). According to the literature the phytic acid is predominantly localized in the quinua embryo (Chauhan *et al.*, 1992). Phytic acid P also is present in many cereals as barley (60%) and rye (61 %), and legumes (Eeckhout and De Paepe, 1994).

The phytase activity was highest in QC (1052 FTU/kg) with significant differences (P< 0.05) between QBA (593 FTU/kg), QB (613 FTU/kg) and QP (561 FTU/kg), as well as was found in the wheat control (1046 FTU/kg) (Table 4).

Phytase activity of the quinoa grain was investigated because of its lack of literature on this subject. This study found that quinoa presented a high endogenous phytase activity. The phytase activity estimated in the QC variety (1052 FTU/kg) was similar with wheat control. The differences reported in phytase activity in this study between the variety QC and QB, QBA and QP may be as result of different desaponification processes (liquid craft and industrialized). Studies by Lorenz and Nyanzi (2007) of total amylase, cellulase and hemicellulase activities reported to be the highest in unprocessed quinoa seeds. The enzymatic activities decreased after mechanical abrassion of seeds or heat treatment after saponin removal caused a further decrease in activity of these enzymes (Lorenz and Nyanzi, 2007).

We found a negative correlation (range $r^2$ between -0.79 and -0.89) between the phytase activity and the amount of phytate P for all varieties. Phytase enzyme is highly effective in catalyzing phytic acid dephosphorylation (Berry *et al.*, 2007), reduction in the content up to 84% (Centeno *et al.*, 2001). Phytases have been studied intensively in recent years, because of the great interest, due to functionality in reducing phytate content in food for human consumption and animal feed (Konietzny and Greiner, 2004).

**Table 4.** Phytic acid P (g/kg total P) and phytase activity of quinoa grain[1]

| Variety | Total P (mg/g DM) | Phytic acid P (mg/g DM) mean±SD[3] | phytic acid P/Total P % | Phytase activity (FTU/kg) mean± SD |
|---|---|---|---|---|
| QC[1] | 4.97 | 0.979 ± 0.003 | 19.64c | 1052,10±77.70a |
| QBA | 4.50 | 1.433 ± 0.024 | 26.95b | 593.30 ±61.62c |
| QB | 4.44 | 1.859 ± 0.059 | 33.60a | 613.20±103.60b |
| QP | 5.08 | 1.941± 0.033 | 32.17a | 560.80±153.53d |
| [2]Wheat | 3.33 | 2.008± 0.023 | 60.22 | 1046.86±170.5 |

[1]QC: Nariño variety from Colombia; QBA: Commercial from Bolivia: Anapqui's quinoa; QB: Commercial -IICA - 020 – Oruro from Bolivia; QP: Huancavelica variety from Peru. [2]Wheat is the control sample. *Phytic acid contains 28.15% P. [3] Means ± SD. Means followed by the same letter are not significantly different at 95% confidence limits.

## Conclusion

- These results showed that the quinoa has high concentration in amino acid and fatty acid profile. In addition we found phytase activity and a negative correlation with phytic acid P content in quinoa. The low level of phytic acid P and high phytase activity in four varieties, demonstrates the importance of these components of quinoa for human nutrition.

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## References

Ando, H.; Chen, Y.; Tang, H.; Shimizu, M.; Watanabe, K. and Mitsunaga, T. (2002). Food components in fractions of Quinoa seed. Food Sci Technol Res. 8 (1): 80-84.

Berry, D.; Shang, Ch.; Waltham, C. and Zelazny, L. (2007). Measurement of phytase activity using tethered phytic acid as an artificial substrate: Methods development. Soil Biol Biochem. 39: 361-367.

Bhattacharya, A.; Banu, J.; Rahman, M.; Causey, J. and Fernandes, G (2006). Biological effects of conjugated linoleic acids in health and disease. J Nutr Biochem. 17: 789-810.

Comai, S.; Bertazzo, A.; Bailoni, L.; Zancato, M.; Costa, C. V. L. and Allegri, G. (2007). The content of proteic and nonproteic (free and protein-bound) tryptophan in quinoa and cereal flours. Food Chem. 100: 1350-1355.

Cardozo, A. and Tapia, M. (1979). Valor nutritivo. In Tapia, M., Gandarillas, H., Alandia S., Cardozo A., Mujica A. Quinua y Kañiwa, Cultivos Andinos. Bogota CIID, Oficina Regional para la America Latina, 1979. p. 149-192. ISBN: 0-88936-200-9.

Centeno, C.; Viveros, A.; Brenes, A.; Canales, R.; Lozano, A. and De La Cuadra, C. (2001). Effect of several germination conditions on total P, phytate P, phytase, and acid phosphatase activities and inositol phosphate esters in rye and barley. J Agric Food Chem. 49 (7): 3208-3215.

Corredor, G. (2003). Proyecto quinua. In: Corredor, G., Romero, A., Ochoa, M. T. (Eds), memorias del curso producción de Quinua cultivo multipropósito. Bogotá, Colombia: Universidad Nacional de Colombia, 33p.

Chauhan, G. S.; Eskin, N. A. M. and Tkachut, R. (1992). Nutrients and antinutrients in Quinoa seed. Cereal Chem. 69 (1): 85-88.

Cheeke, P. R. (l995). Antinutritionals factors in legume seeds. *Book reviews* /Anim Feed Sci Technol. 5l: 337-343.

Doyle, L.; Jewell, C.; Mullen, A.; Nugent, A P.; Roche, H M. and Cashman, K D (2005). Effect of dietary supplementation with conjugated linoleic acid

on markers of calcium and bone metabolism in healthy adult men. Eur J Clin Nutr. 59: 432-440.

Dušková, D.; Marounek, M. and Březina, P. (2001). Determination of phytic acid in feeds and faeces of pigs and poultry by capillary isotachophoresis. J Sci Food Agr. 81: 36-41.

Eeckhout, W. and De Paepe M (1994). Total phosphorus, phytate-phosphorus and phytase activity in plant feedstuffs. Anim Feed Sci Technol. 47: 19-29.

FAO (1989). Utilization of tropical foods: Cereals. Food and Nutrition Paper. 47/1, pp 109-112.

Improta, F. and Kellems, R. O. (2001). Comparison of raw, washed and polished quinoa (*Chenopodium quinoa* Willd.) to wheat, sorghum or maize based diets on growth and survival of broiler chicks. Livest Res Rural Dev. 13 (1), 10 p.

ISO 5509 (2000). Animals and vegetal fats and oils. Preparation of methyl esters of fatty acids. Geneva, Switzerland: International Organization for Standardization.

Konietzny, U. and Greiner, R. (2004). Bacterial phytase: potential application, in vivo function and regulation of its synthesis. Braz J Microbiol. 35: 11-18.

Koziol, M. J. (1993). Quinoa: A potential new oil crop. In: J. Janick and J.E. Simon (Eds.), New crops, Wiley, New York, USA: FAO, pp. 328-336.

Kyriakidis, N. B.; Galiotou-Panayotou, M.; Stavropoulos, A. and Athanasopoulos, P. (1998). Increase in phytase activity and decrease in phytate during germination of four common legumes. Biotechnol Lett. 20 (5): 475-478.

Lorenz, K. and Nyanzi, F. (2007). Enzyme activities in quinoa (*Chenopodium quinoa*). Int J Food Sci Tech. 24(5): 543-551.

Lei, X. G.and Porres, J. M. (2003). Phytase enzymology, applications, and biotechnology. Biotechnol Lett 25: 1787-1794.

OAC (1980). Official Methods of analysis, Washinton, DC, USA: Association of Official Analytical Chemists.

Park, S. H. and Morita, N. (2004). Changes of Bound of Quinoa Seeds Lipids and Composition of Fatty Acids in Germination. Food Sci Technol Res. 10 (3): 303-306.

Prakash, D. and Pal, M. (1998). Chenopodium quinoa: Changes in amino acid composition in seed during maturity. Int J Food Sci Nutr. 49 (4): 285-288.

Marounek, M. (2010). Význam kyseliny fytové ve výživě zvířat a lidí. In Opletal, L., Skřivanová, V. Přírodní látky a jejich biologická aktivita. 2. Využití látek pro ovlivnění fyziologických procesů hospodářských zvířat, Nakladatelství KAROLINUM. Universita Karlova, pp. 175-202.

Marounek, M.; Skřivan, M.; Dlouhá, G. and Břeňová, N. (2008). Availability of phytate phosphorus and endogenous phytase activity in the digestive tract of laying hens 20 and 47 weeks old. Anim Feed Sci Technol. 146: 353-359.

Mendoza, G. (1993). Alternativas de producción y consumo de quinua en Colombia. Bogotá, Colombia: ICBF, Instituto Colombiano de Bienestar Familiar.

MINITAB 2006. Statistics version 15. Minitab Inc. State College, Pennsylvania, Philadelphia, EE.UU.

Mujica, A.; Izquierdo, J. and Marathee, J-P. (2001). Quinua y la kañiwa: cultivos andinos. Capitulo I. Origen y Descripcion de la Quinua. CIID. Oficina Regional para América Latina y el Caribe, FAO: Santiago de Chile, [CDROM Version 1].

Reddy, N. R. and Sathe S K (2002). Food phytates. Boca Raton, Florida, USA: CRC PRESS.

Reddy, N. R.; Pierson, M. D.; Sathe, S. K. and Salunkhe, D. K. (1989) Phytates in cereals and legumes. Boca Raton, Florida, USA: CRC PRESS

Ruales, J. and Nair, B. M. (1993). Content of fat, vitamins and minerals in quinoa (*Chenopodium quinoa*, Willd) seeds. Food Chem. 48: 131-136

Ruales, J. and Nair, B. M. (1993). Saponins, phytic acid, tannins and protease inhibitors in quinoa (*Chenopodium quinoa*, Willd) seeds. Food Chem 48, 137-143.

Sokal and Rohlf, (1995). Biometry: The Principles and Practices of Statistics in Biological Research. Edition: 3rd. ISBN-13: 978-0716724117.

Suchner, U.; Kuhn, K. S. and Fürst, P. (2000). The scientific basis of immunonutrition. Proc Nutr Soc. 59: 553-563.

Thanapornpoonpong, S-N.; Vearasilp, S.; Pawelzik, E. and Gorinstein, S. (2008). Influence of various nitrogen applications on protein and amino acid profiles of amaranth and quinoa. J Agric Food Chem. 56 (23): 11464-11470.

Wright, K. H.; Pike, O. A.; Fairbanks, D. J. and Huber, C. S. (2006). Composition of Atriplex hortensis, Sweet and Bitter Chenopodium quinoa Seeds. J Food Sci. 67 (4): 1383- 1385.