

Argentine Buckwheat Variety: Proximal Analysis, Mineral Content, Antinutritional Factors and Antioxidant Activity

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Abstract – Buckwheat is a pseudocereal, which has gained interest in recent years based on its biophysiological compounds. Since buckwheat is becoming popular nutrition source worldwide alternative, in Argentina has been working in obtaining new experimental line of buckwheat produced in the central region of the country in order to promote its use. The goal of this present work was to determine the proximal analysis, mineral content, antinutritional factors and antioxidant compounds and evaluate their activity in a new Argentine buckwheat called ALMNO13. Proximal Analysis was: moisture $11.5 \text{ g } \% \pm 0.26$, ash $2.0 \text{ g } \% \pm 0.29$; proteins $14\% \pm 0.18$; fat $6.1 \text{ g } \% \pm 0.19$, fiber $15\% \pm 0.40$, carbohydrates $51.5 \text{ g } \%$, caloric value 316 kcal. Mineral content was Cu: $5.40 \text{ mg}/100\text{g} \pm 0.19$; Zn: $21.72 \text{ mg}/100\text{g} \pm 1.93$; Fe: $25.48 \text{ mg}/100\text{g} \pm 4.23$; Ca: $39 \text{ mg}/100\text{g} \pm 0.77$; K: $19.96 \text{ mg}/100\text{g} \pm 11.66$; Mg: $22.55 \text{ mg}/100\text{g} \pm 10.76$; Mn: $16.35 \text{ mg}/100\text{g} \pm 0.74$; P: $16.70 \text{ mg}/100\text{g} \pm 0.9$; Cr: $0.37 \text{ mg}/100\text{g} \pm 0.06$; Se: $0.022 \text{ mg}/100\text{g} \pm 0.7$. The presence of antinutritional factors (saponins, oxalates, nitrates, phytates, lectins and trypsin inhibitors) was determined and resulted within the acceptable values for human consumption or were negative. Total phenols, flavonoids and antioxidant activity evaluated were: $133.5 \pm 0.78 \text{ mg}$ of gallic acid equivalent/100g in dry weight; $24.80 \pm 0.89 \text{ mg}$ quercetin equivalent/100 g dry weight. The antioxidant activity was estimated by DPPH radical scavenging activity and nitric oxide scavenging activity were: 83.70 ± 1.86 and 48.02 ± 0.78 respectively. These results show that the Argentine experimental line ALMNO13 buckwheat is safe for human consumption and can be used as an ingredient in food, contributing in the nutritional quality of the diet. This is the first report on the composition and antioxidant activity of this buckwheat new variety.

Keywords – Buckwheat New Line, Proximal Analysis, Antinutritional Factors, Minerals Content, Polyphenols, Flavonoids, Antioxidant Activity.

I. INTRODUCTION

The promotion of minor crops cultivation is in accordance with the commitments of sustainable agriculture emphasized in EASAC Policy Report (2011) ensuring eco-efficient production, more nutritious and quality foods and minimizing land use and inputs. Furthermore, these crops may represent an economically sustainable mean for the biodiversity enhancement (1;2). Among minor crops, buckwheat (*Fagopyrum* spp.), a pseudo-cereal belonging to the Polygonaceae family, has received increasing interest. Minor crops (buckwheat, oat, millet, rye, etc.) are grown in limited areas or produced in small quantities, and have the main limitation to wider utilization in their reduced grain yield potential. The development of improved cultural practices, together with the creation of more productive cultivars, can afford to increase the yield and the quality of these crops, allowing full exploitation and use, thus offering to consumers the tools to take care of their health in a simple and cost-effective way. Among minor crops, buckwheat (*Fagopyrum* spp.), a pseudocereal belonging to the Polygonaceae family has received increasing interest thanks to the growing evidence of beneficial properties of some grain components on health (3;4).

In recent years, interest in buckwheat has been renewed as an alternative crop for organic farming and as healthy foods (5). The buckwheat belongs to Polygonaceae family, is a pseudocereal, whose origin in China, used in human food since ancient times. There are approximately 19 species, in addition to the wild varieties, although the most commonly used are common buckwheat (*Fagopyrum esculentum* Moench) and tartaric buckwheat (*Fagopyrum tartaricum* (L.) Gaertn). Its short growing season and its ability to thrive in soils made buckwheat an accessible option for most of the agricultural and poor population of Europe. Upon entering America, he enjoyed great popularity during the 19th century. Buckwheat has great potential as a food ingredient, especially for the functional food industry, because it does not contain gluten, as nutritional stuff it contains proteins of high biology value, dietary fiber, resistant starch, D-chiro-inositol, phagopyritol, vitamins and minerals. Starch and fiber are present in similar amounts of cereals and buckwheat also contains a high level of polyunsaturated essential fatty acids such as linoleic acid. Several vitamins (B1, C, and E) are present, whereas minerals are present in abundance (6).

Buckwheat grain can be proposed as a functional ingredient due to the richness of bioactive compounds and, moreover, being gluten free can be recommended for celiac disease-affected people, (7). Buckwheat grain is rich in flavonoids so the introduction of its flour in the recipes of traditional foods products (eg, bakery products and pasta) has led to innovative and potential functional foods (8). The regular intake of buckwheat cookies may favor the reduction of myeloperoxidase levels, an indicator of inflammation, and may lower serum cholesterol levels (9).

Antinutrients can be defined as substances naturally present in foods, which by themselves or through their metabolites generated by the organism, alter the use of nutrients, interfering in metabolic processes or in their bioavailability, being able to affect the health consumers. Anti-physiological compounds are also found in the grains, such as phytates, digestive enzyme inhibitors, hemagglutinins, phenols and tannins. The antinutritional factors can be classified as thermostable or thermolabile factors; among the first are: saponins, oxalates, nitrates, phytates, cyanogens, antigenic factors, oligosaccharides, and toxic non-protein amino acids; among the thermolabile factors are: lectins, proteases inhibitors (trypsin and chymotrypsin) and antivitamin. They can also be classified according to their mechanism of action into: mineral sequesters, which act by interfering with the mineral's assimilation (saponins, oxalates, phytates, anti-thyroid factors, ovotransferrin); antivitamin, which inactivate or increase the vitamins requirements (anti-thiaminases, ascorbic oxidase, avidin); and antienzymes, which inhibit the action of endogenous digestive enzymes (proteases inhibitors, amylases) (10). Despite the antinutritional factors negative effects, the scientific evidence has demonstrated their beneficial role in the prevention and treatment of chronic- degenerative diseases, when found in adequate amounts that do not affect health.

Numerous evidences from epidemiological studies have shown that there is a significant correlation between the increase in the consumption of fruits, vegetables and grains, with the decrease in the incidence of coronary diseases, some common types of cancer, diabetes, arthritis and other degenerative diseases. This is attributed to the fact that these foods provide phytochemical compounds, mainly natural antioxidants. The antioxidant properties of polyphenols have been extensively studied, and it has become clear that the mechanisms of action of polyphenols go beyond the modulation of oxidative stress (11).

These antioxidants such as phenolic compounds, flavonoids, some vitamins and minerals, are significant constituents of the non energetic part of human diets naturally found in vegetables. They exert multiple biological effects, due to their antioxidant and free radical-scavenging abilities, and are potentially beneficial for health, as they inhibit lipid peroxidation. The antioxidant activities of these phenolic compounds are strongly correlated with a variety of phytochemical contents in buckwheat. The improved antioxidant activities of buckwheat phenolic compounds play the key role of a fundamental prophylactic property for human health; thus, buckwheat seed can be a great functional food source for human health promotion. The rise in nutritional quality and functionality happens during the crop seed germination. Various phenolic compounds including phenolic acid, rutin, and C-glycosyl flavones are accumulated in buckwheat seeds. The nutritional effect of buckwheat seed shows strong antioxidant activities as well as various chemical compositions over 72 h of seed germination (12). Some authors have demonstrated nutraceutical properties of the pseudocereals grains, such as antihyperlipidemic, antidiabetic and hypocholesterolemic activity, as well as its antioxidant activity. (13;14). Although dietary phytochemicals play a major role in regulating human health and disease (15), further attempts could be made concentrating research activities on a pool of biomolecules contained in buckwheat grains (digestible starch, protein, vitamins, minerals, and amino. The goal of this present work was to determine the proximal analysis, mineral content, antinutritional factors and antioxidant compounds and evaluate their activity in a new Argentine Buckwheat called ALMNO13, obtained in experimental plots; this becomes important considering that the new line studied in this work have agronomic advantages that the crop and the resistance towards some pathogens. In order to promote its use in human diet, since

buckwheat is becoming an increasingly popular nutrition source worldwide alternative.

II. MATERIALS AND METHODS

Sample and Reagents

Work was performed on buckwheat advanced lines (ALMNO13) seeds, from experimental crops of the Faculty of Agronomy and Veterinary of the National University of Río Cuarto, Province of Córdoba, Argentina (experimental crops from 2012 vintage).

Raw seeds were dried in a forced air oven, at 45°C for 48 h. The dried product was ground in an electric coffee grinder (CG-8 Stylo, 220 V-50 Hz 90 W, China) and sieved through a 200-µm nylon sieve and conserved in a hermetically sealed container, protected from light, and stored at 4°C. All reagents used were of analytical grade. The analyses were performed in triplicate and the mean value of dry matter was obtained.

Proximal Analysis

Moisture, protein, fat, ash, fiber contents were determined using the AOAC methods (925.10, 945.39, 978.04-968.01, 962.09, 945.46, (16)). The carbohydrate content (%) was calculated by subtracting the crude ash, fat, fiber and protein content of 100% of the dry matter.

Caloric Value

The caloric value was calculated on the proximal composition using the Atwater factors (Proteins: 4 Kcal / g - Fat: 9 Kcal / g - Carbohydrates: 4 Kcal / g).

Mineral content

Experimental-Reagents- Instrumentation

Concentrated nitric acid (HNO₃, 70%), hydrogen peroxide (H₂O₂, 30%, Merck), ultrapure deionized water obtained from a purification system (Barnsted) were used.

Microwave digestion system (START-D Milestone, Italy), equipped with a temperature display screen, adjustable up to 300 °C and 1200 watts of power and with an operating frequency of 2.5 GHz. It has eight reactors (polytetrafluoroethylene) of 100 mL capacity. Before each digestion, the reactors were cleaned by adding 8 mL of nitric acid (5% v / v) and according to the manufacturer's schedule. For the study of elemental composition, an ICP-MS (SCIEX, ELAN DRC-e, Perkin-Elmer, Canada) was used. The operating conditions of the instrument were 1.35 kW power, argon gas (spectral purity, 99.9998%), 14.00 L / min flow rate (plasma), 1.4 L / min (auxiliary) and 1.2 L / min (nebulizer).

Sample pretreatment

For the determination of temperature, they were dried at 60 °C until constant weight was achieved. The dried samples were homogenized and ground in an agate mill. The ground samples were properly labeled and stored in plastic jars.

Microwave digestion

For digestion, 0.5 g of the buckwheat sample was accurately weighed directly in the digestion vessel, it was carried out in triplicate, followed by the addition of 7.0 mL of concentrated HNO₃ and 2.0 mL of H₂O₂. The heating procedure was with a power of 1000 W up to 200 °C in 10 min and a second stage where it was kept at 200 °C for 10 min. After cooling, the contents of each reactor were diluted to 50 mL with ultrapure deionized water. Reagent blanks were made, which were prepared under the same conditions as the samples.

Antinutrients content

Saponins

Saponins main properties are their ability to produce hemolysis and generate foam, and these characteristics are the ones taken into account for the present determination. Saponins were determined by measuring the hemolytic activity and the foam index (17;18). The hemolytic activity was evaluated using goat blood cells (20 mL of the red blood cells at 2% in physiological solution were added to 30 mg of defatted flour), which were observed for a period ranging from 30 min to 12h; a numerical score was used:

0 (no hemolysis within 12 h), 1 (10% hemolysis within 12 h), 2 (20–

40% hemolysis within 12 h), 3 (50–90% hemolysis within 12 h), 4 (100% hemolysis within 12 h), 5 (100% hemolysis within 30 min). Values of 0–2 were considered to be indicative of low hemolytic activity, and values of 3–5 of high activity. The foam index (FI) was determined by the following procedure: about 1 g of the plant material was reduced to a coarse powder (sieve size No. 1250), weighed and transferred to a 500 mL conical flask containing 100 mL of boiling water. Moderate boiling was maintained for 30 minutes. The solution was cooled and filtered into a 100 mL volumetric flask, and sufficient water was added through the filter to complete 100 mL. The above decoction was placed into 10 stoppered test tubes (16 cm in height, 16 mm in diameter) in a series of successive portions of 1, 2, 3, up to 10 mL, and the volume of liquid in each tube was adjusted with distilled water to 10 mL. The tubes were stoppered and shaken in a lengthwise motion for 15 seconds at 2 Hz. The filtrate solution was allowed to stand for 15 minutes and the height of the foam was measured. The FI was calculated as $1000/a$, where a is the volume in mL of filtrate used for the solutions in which the foam reached 1 cm. When the foam did not reach 1 cm, the FI was reported as <100 .

Oxalates

For the extraction of oxalic acid, the sample was treated with HCl, and then the phosphotungstic reagent was added; the oxalic acid was precipitated as calcium oxalate, which was then dissolved with H₂SO₄, and subsequently quantified with KMnO₄ (19).

Nitrates

Nitrates were determined using the method of Cataldo et al. 1975 (20). This method is based on the formation of a colored complex by nitration of salicylic acid under acid conditions, which is recorded in a spectrophotometer at 412 nm, at alkaline pH (pH > 12). Different concentrations of NaNO₃ were used as controls (starting from a concentration of 1 g/L). The supernatant from the treatment of the sample with bidistilled water and incubated at room temperature was used.

Phytic Acid

The phytate was extracted with trichloroacetic acid and precipitated as a ferric salt; the iron content of the precipitate was determined colorimetrically (510 nm). The phytate content was expressed in terms of phosphorous content, and calculated from the standard curved prepared for this technique, assuming that 4Fe:6P is constant in the precipitate (21).

Lectins (Hemagglutinins)

These proteins have a strong ability to agglutinate the red blood cells of several species in vitro, so that their activity was determined prior to saline extraction, according to the method of Do Prado et al., 1980 followed by the corresponding quantification according to the method proposed by Das Gupta and Boroff, 1968. The analysis was carried out on a microtitre plate by performing a series of 2-fold dilutions, and adding a suspension of red blood cells (Group “O”, Factor Rh +). The reading was performed considering the minimum protein concentration capable of producing a positive haemagglutination reaction. (22; 23).

Antitryptic Activity

The antitryptic activity is based on the determination of the protease activity decrease provoked by an extract that contains the inhibitors (24). The extraction was performed with NaOH 0.01 N from defatted flour, with continuous agitation until reaching a suspension pH of between 9.5 and 9.8; it was then centrifuged, and the enzymatic test was performed on an aliquot of the supernatant using BAPA (benzoyl-DL-arginine-p-nitroanilide hydrochloride) as substrate, and trypsin as enzyme. It was spectrophotometrically measured at 410 nm.

Extraction of Total Phenols

The flours were defatted and the lipid extraction was performed by refluxing the samples in hexane for 10 hours using a Soxhlet apparatus. The hexane was evaporated and the samples were stored at 5°C. The extraction of total phenols was carried out from 50 mg of defatted sample with 5 mL of 1.2 mol/L HCl in 50% methanol: water. The sample was heated at 90°C for 3 h with vortexing every 30 min. Subsequently, the sample was cooled and diluted to 10 mL with methanol, and centrifuged for 5 min at 5000 g. The supernatant was used for the determination of phenols, flavonoids, anthocyanins and antioxidant activity (25).

Total Phenols

The determination of total phenols was measured at 750 nm using the Folin-Ciocalteu reagent with gallic acid as a standard, and it was expressed as mg of gallic acid equivalent/100 g dry weight. Aliquots of 0.5 mL of standard, distilled water (blank) and methanolic extract, were added to flasks containing 4.5 mL of distilled water; afterwards, they were mixed with 0.5 mL of the Folin-Ciocalteu reagent and 5 mL of 7% sodium carbonate, and the resulting solutions were taken to 12.5 mL with distilled water. The mixtures were allowed to stand for 90 min at room temperature before measuring their absorbance at 750 nm (UV-Vis Beckman DK-2^a) (26).

Flavonoids

The content of total flavonoids was determined by a colorimetric method. AlCl₃ was used as complexing agent. First, 0.25 mL of the sample extract were diluted with 1.25 mL of distilled water; then, 75 µL of a 5% NaNO₂ solution was added to the mixture. After 6 min, 150 µL of a 10% AlCl₃·6H₂O solution was added, and the mixture was allowed to stand for 5 min. Subsequently, 0.5 mL of 1.0 mol/L NaOH was added, and the resulting solution was taken to 2.5 mL with distilled water. The solution was well-mixed, and the absorbance was immediately measured against the prepared blank at 510 nm using a spectrophotometer (UV-Vis Beckman DK-2^a), and then compared with the standards prepared likewise with known catechin concentrations. The result was expressed as mg of catechin equivalent/100 g dry weight (27).

Antioxidant Activity

The antioxidant activity of the ALMNO13 grains extract was assessed by the following methods. Tests were performed in triplicate.

DPPH Radical Scavenging Activity (DPPH Assay)

This spectrophotometric assay uses the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) as reagent. This method relates the sample capacity to inhibit the action of free radicals generated by DPPH, in a highly polar environment and in the absence of an oxidizable lipid. The hydrogen atom or electron donor ability of the corresponding extract was measured from the bleaching of a purple-colored MeOH solution of DPPH (28). Various concentrations of the extract (2.5 mg/mL) in MeOH (50 µL) were added to 5 mL of a 0.004% MeOH solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was measured against a blank at 517 nm (UV-Vis Beckman DK-2^a). The blank contained all reagents except for the tested compound. The synthetic antioxidant butylated hydroxy toluene (BHT) was included in the experiments as a positive control (1.6 µg/mL). The percentage of DPPH inhibition was calculated using the following equation:

$$\% \text{ DPPH Inhibition} = (A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}) \times 100$$

A = Absorbance

Nitric Oxide Scavenging Activity (NO Test)

Nitric oxide was generated from sodium nitroprusside and measured by the Griess reaction (29). Sodium nitroprusside in aqueous solution and at physiological pH, spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated by using the Griess reagent, with which nitrite reacts to give a stable product that absorbs at 542 nm. Scavengers of nitric oxide compete with oxygen leading to a reduced production of nitrite. The sodium nitroprusside solution was prepared immediately before the experiment by dissolving 10 mmol/L sodium nitroprusside in 0.02 mol/L phosphate buffer (pH 7.4) previously bubbled with argon. The samples were diluted in 0.02 mol/L phosphate buffer to obtain optimal concentrations. At the beginning of the experiment, an aliquot of 1 mL of the samples (2.5 mg/mL) was diluted with 1 mL of sodium nitroprusside solution, and incubated at room temperature for 150 min. At the end of the incubation period, 2 mL of Griess reagent (1% sulphanilamide, 2% H₃PO₄ and 0.1% naphthylethylenediamine dihydrochloride) were added to each sample, and the absorbance was measured at 542 nm (UV-Vis Beckman DK-2^a). A control without extract, but with an equivalent amount of methanol, was conducted in an identical manner. The results were expressed as percentage

(%) of nitrite production inhibition.

$$\% \text{ Nitrite Inhibition} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

A = Absorbance

III. RESULTS AND DISCUSSION

In this study, was determined the proximal composition, mineral content, antinutritional factors, and the antioxidant activity of the new advanced lines of Argentine buckwheat called ALMNO13. Table1 shows the proximal composition (g/100g): moisture 11.5, ash 2.0, protein, 14, fat 6.1, fiber 15; carbohydrates 51.5, caloric value 316 kcal.

Table 1. Proximate composition from ALMNO13 buckwheat seeds

Determination (%)	(g/100g)
Moisture (MF) ^a	11,5 % ± 0.26
Ash	2.0 % ±0.29
Protein (N x 6.25)	14 % ± 0.18
Total fat	6.1 g % ± 0.19
Total fiber	15 % ± 0.40
Total Carbohydrates ^b	51.5 g %
Caloric value	316 kcal ± 0,06
a fresh basis	
b calculated as 100- (% moisture + % protein+ % fat + % ash+ % fiber)	

* Results are expressed as mean ± standard deviation (n = 8). Different letters indicate significant differences (p<0.05).

Results for protein and fat found to be in range reported by Pandey et al. 2015. Fiber and ash contents were found to be higher than recorded by (31;7;32) but a slightly difference to the reported by Hatcher et al. 2008, from cross- and self-pollinating Canadian common buckwheat had (15.2-22.0%) of total fiber content. (30;33).

The ash content of buckwheat varies may vary from 2.0-2.2%, depending upon the variety and conditions during growth (34;7).

The differences in protein content from several buckwheat cultivars may be due to cultivars variability and growing conditions. Wei et al., 2003 registered 13.30-15.55% protein content in most buckwheat grains and only a different percentage of the 10.3% was reported in common buckwheat. (35; 36; 37;38).

Moisture content was in range reported to maintain the storage life of mostly cereals. Slight difference in composition from previous record might be due to difference in climatic conditions of crops and environment in which experiments were conducted Raghuvanshi et al, 2017. (39).

The content of certain important minerals in pseudocereals as previously published by Alvarez- Jubete et al., 2009. A number of authors have also highlighted the exceptional mineral content of these seeds (41; 42; 43). The nutritional functions of minerals in buckwheat and foods prepared from it have been studied by many scientists (44; 45; 46; 47; 48; 49). Calcium, magnesium and iron are minerals that are deficient in gluten-free products and in the gluten-free diet (50; 51; 52; 53). The pseudocereals are generally a good source of these and other important minerals (40).

Table 2 shows the concentrations (mg / g): Cu: 5.40 mg/100g ± 0.19; Zn: 21.72 mg/100g ± 1.93; Fe: 25.48 mg/100g ± 4.23; Ca: 39 mg/100g ± 0.77; K: 19.96 mg/100g ± 11.66; Mg: 22.55 mg/100g ± 10.76; Mn: 16.35 mg/100g ± 0.74; P: 16.70 mg/100g ± 0.9; Cr: 0.37 mg/100g ± 0.06; Se: 0.022 mg/100g ± 0.7. In comparison with rice, wheat, corn, buckwheat contains highest amounts of zinc, copper, iron, magnesium and manganese. For Calcium ALMNO13 have similar amounts to cereals and common, Tartary and Hungarian buckwheat seeds. Selenium, was present in ALMNO13 buckwheat in a similar level than Hungarian buckwheat. Chromium and phosphorus are reported in these new grains. (4;54;55).

Compared with others buckwheat species, the new buckwheat contains higher levels of zinc (Zn), copper (Cu) and manganese

(Mn). All of these studies concluded that buckwheat grains are a good source of many essential minerals (56).

Table 2. Mineral content from ALMNO13 buckwheat seeds

Determination	(mg/100g dw)
Cu	5.40 ± 0.19
Zn	21.72 ± 1.93
Fe	25.48 ± 4.23
Ca	39±0.77
K	19.96 ± 0.6
Mg	22.55 ± 10.76
Mn	16.35 ± 0.74
P	16.70± 0.9
Cr	0.37 ± 0.6
Se	0.022 ± 0.7
Data presented as mg/100 g dry weight basis standard deviation.	

Antinutrients content

In order to achieve a better use of the nutritional potential of these seeds, it is important to identify the antinutritional factors that could affect their bioavailability. In this work, thermostable and thermolabile factors were studied among the first: saponins, oxalates, nitrates and phytates.

Saponins are steroids or triterpenoid glucosides that, to a great extent, determine the bitter taste of some seeds; they have common properties that are used for their determination in foods, such as their surfactant activity in aqueous solutions and their hemolytic action. Saponins are not absorbed in the intestine, and therefore affect the absorption of zinc and iron. However, many studies indicate that saponins have a wide range of biological activities and beneficial effects, such as their immunostimulant, antifungal, antiviral, anticancer, hypocholesterolemic and hypoglycemic action, among others (57). The foam index analyzed in the samples gave a result of <100, indicating low activity, and hemolysis was not observed during the studied 12-hour; both results suggest a low concentration of saponins (Table 3). In other studies, performed in grain buckwheat varieties (58), it was concluded that the buckwheat saponins, due to their low content and low toxicity, are not a significant risk for the consumer.

Table 3. Thermostable antinutritional factors from ALMNO13 buckwheat seeds

Determination	(mg/100 g)
Foam Index a	<100
Hemolytic Activity	negative
Oxalic acid	3.92 ± 0.47a
Nitrates	109.25 ± 1.08a
Phytic Acid	50.03 ± 1.10a (as P)

The results are expressed as mean ± standard deviation of triplicate determinations. Different letters indicate significant differences ($P < 0.05$).

^a1000/a; a = amount of filtrate (in ml) in the tube that reached 1 cm of foam. Since no tube exhibited 1 cm of foam, FI < 100.

Oxalates are present as sodium oxalate in plants, and are mainly accumulated in the leaves; this compound is soluble, and combines with calcium and magnesium in the bloodstream, resulting in insoluble salts (calcium oxalate and magnesium oxalate). These salts, when present in large amounts in human tissues, can lead to damage by oxidation and glutathione depletion, and can also generate cascade inflammation by an immunological effect and the formation of kidney stones (59). The amount of oxalic acid found in the seeds under study varies from 3.92 /100 g of sample (Table 3). Siener et al., 2006 determined the content of total soluble oxalate in several types of cereals (rice, wheat, rye, oat, barley and corn), finding that it varies from 3.60 to 376.60 mg/100 g (60). For these authors, the oxalate in the whole grain is higher in comparison to the refined products, which suggests that the oxalic acid is mainly at the external layers of the cereals. On the other hand, the American Dietetic Association's Nutrition Care Manual instructs patients with kidney stones to follow an oxalate-restricted diet in amounts lower than 40 to 50 mg per day. Taking this into account, our results are low and within the suggested limits.

The Joint FAO/WHO Expert Committee has determined as Acceptable Daily Intake (ADI) of nitrates a value of 0 - 3.7 mg/kg of body weight, expressed in nitrate ions, which is equivalent to 222 mg of nitrate for an adult of 60 kg in body weight (61). The presence of nitrates in seeds is a consequence of the nitrogen cycle, in which the plant assimilates inorganic nitrogen in the form of nitrates, to use them in the synthesis of vegetable proteins. The nitrates problem lies on that they can be reduced to nitrites, this biotransformation to nitrites is performed by the intestinal flora. Nitrites produce the transformation of hemoglobin to methemoglobin, which reduces the amount of oxygen transported in the blood. Furthermore, once the nitrites are formed, they can react with amines, which are substances widely present in our organism, originating nitrosamines, a type of compound with carcinogenic action (62). The nitrates content in ALMNO13 Buckwheat presents nitrates from 109.25 ± 1.08 (mg/100 g) as shown in Table 3. Taking into account the nitrate levels found in ALMNO3 buckwheat seeds has the recommended nitrate limit.

Phytic acid is the hexaphosphoric ester of cyclohexane. In general, it is present in foods at neutral pH, and is a molecule with a negative charge, therefore, very reactive. Phytic acid has a strong sequestering action of several important minerals such as calcium, magnesium, iron and zinc. When a mineral binds to phytic acid, it becomes insoluble, precipitates and will not be absorbed in the intestine; it also decreases the absorption of niacin and proteins (63). Phytates are not only considered as antinutrients, but also, some recent studies show that the less phosphorylated forms decrease the glycemic index through the action of endogenous phytases during digestion, since they inhibit the action of amylases (63), is also a natural constituent possessing moderate anticancer activity thus preventing colon cancer (64). In addition, they act as antioxidants, since they inhibit the peroxidase and prevent the formation of kidney stones, reducing the formation of hydroxyapatite crystals (65). The values founded in this work was 50.03 ± 1.10 mg of P/100g of flour (Table 3). Other authors obtained similar results for diverse species of pseudocereals (52mg /100g) and in comparison, the obtained value was lower than complete cereal like wheat (170 mg/100 g) (66). Among the thermolabile factors, lectins and trypsin inhibitors were determined. Lectins belong to a diverse group of non-immune proteins known as hemagglutinins, which are widely distributed in nature. Lectins from food can be detected using their *in vitro* function, they combine with the membrane glycoproteins of red blood cells causing their agglutination. The *in vivo* action of lectins can affect the nutrients absorption and transport processes through small intestine due to the high specificity to recognize intestinal glycoproteins. Several studies show that lectins have biological properties such as mitogenesis, promotion of cellular adhesion, inhibition of mycotic growth and insulin-mimetic action, so that they can be used in research techniques. Between other beneficial effects of these compounds can mention, inhibit tumor growth with a similar prebiotics effects, since they produce an increase of the fermentable matter in the colon (67). As shown Table 4, the hemagglutinating activity of ALMNO13 seeds was 1/64, in agreement with the results of others pseudocereals values, and do not affect health, considering that the lectins activity is reduced or eliminated by the action of heat (68). These results indicate that this new variety of pseudocereal have an adequate nutritional chemical composition (proteins, lipids, minerals, antinutritional factors and total fiber) to be an interesting nutritional source.

Table 4. Thermolabile antinutritional factors from ALMNO13 buckwheat seeds

Determination	
Hemagglutination	1/64
Antitryptic Activity TIU/mg flour ^a	2.85 ± 0.14a
Antitryptic Activity TIU/mg protein ^b	18.00 ± 1.03a

The results are expressed as mean ± standard deviation of triplicate determinations. Different letters indicatesignificant differences ($P < 0.05$).

^aTIU/mg flour = trypsin inhibited units per milligram of flour.

^bTIU/mg protein = trypsin inhibited units per milligram of protein.

Antioxidant activity- Total Phenols

Antioxidant activity is an important prophylactic property for human health (antimutagenic, anticarcinogenic and antiaging, etc.) (69).

Many studies have now confirmed that exogenous antioxidant, especially supplied by foods, are essential for counteracting oxidative stress. These antioxidants come mainly from plants in the formof phenolic compounds (flavonoids, phenolic acids), ascorbic acid and carotenoids. Dietary natural antioxidants have become a major industrial and scientific research challenge over the last 20 years. Several studies shown the fruits, vegetables, cereals and pseudocereals consumption with low risks of get diseases associated to oxidative stress, due to the antioxidants present, which show a great capacity of scavenging free radicals that cause the oxidative damage (70).

The reason why buckwheat has been considered a healthy food is relatedto the strong antioxidant activity of buckwheat grains. This benefit is because of the functional metabolites, like phenolic compounds play key roles in antioxidant activities a fundamental prophylactic property for human health, thus buckwheat seed can be a great functional food source for human health promotion (12;71).

The concentration of total phenolic is shown in Table 5. The studied buckwheat ALMNO13 showed a content of total phenols 133.5±0.78 mg of gallic acid equivalent/100g minor amount with commonbuckwheat (476 GAE/g) but in comparison with wheat, barley, oat, triticale, rye contain higher number of polyphenolics (37 and 130 mg GAE/g extract (69;72; 73; 74; 75; 76).

Table 5. Contents of total phenol, flavonoids from new line ALMNO13 buckwheat seeds

Determination	(mg/100g)
Total Phenols	133.5±0.78 mg a
Flavonoids	24.80 ± 0.89 b

The results are expressed as mean ± standard deviation of triplicate determinations. Different letters indicate significant differences ($P < 0.05$). Expressed as mg Gallic acid equivalent/100 g dry weight. Expressed as mg quercetin equivalent/100 g dry weight.

The variety of buckwheat seed act as important dietary antioxidant, and it might be an alternative to replace synthetic antioxidants as additives in food, pharmaceutical and cosmetic preparations.

Lin et al., 2009, proved antioxidant activity by reducing power and DPPH radical scavenging abilityin buckwheat enhanced wheat bread. Similarly, Sedej et al., 2011 proved that buckwheat flours exhibited significantly higher ($P < 0.05$) antiradical activity

on hydroxyl (OH), superoxide anion (O₂[•]) and DPPH radicals, antioxidant activity and reducing power in comparison with wheat fractions. (77;78).

Flavonoids are phenolic compounds that represent substantial constituents of the non-energetic part of the human diet and also act as strong antioxidants protecting lipids, DNA, proteins and lipoproteins (79;80;81;82). The average intake of these compounds is approximately 23 mg/day (83); thus, a 100g or 50 g daily intake would fulfill daily flavonoids requirements. Flavonoids are efficient antioxidants due to their iron and other transition metals chelation capacity, as well as their radical-scavenging activity. Therefore, they play an essential role in the protection against oxidative damage phenomena and have therapeutic effects on a large number of pathologies, such as ischemic cardiopathies, atherosclerosis and cancer (84). There is evidence that flavonoids could protect membrane lipids from oxidation (85). Moreover, it has been demonstrated that they have a strong capacity to inhibit in vitro oxidation of low-density lipoproteins (LDL), reducing oxidated LDL cytotoxicity (86).

Many studies have suggested a protective role of dietary flavonoids against coronary heart diseases and possibly cancer (87). In recent years, flavonoids have attracted increasing interest because they have various beneficial health effects such as anti-allergic, antiviral, anticancer and anti-oxidation properties (87;88). Flavonoids are known for their effectiveness in reducing cholesterol levels in the blood, keeping capillaries and arteries strong and flexible, reducing high blood pressure and reducing the risk of arteriosclerosis (89;90;91) quantified 32 free and 24 bound phenolic compounds in buckwheat flour and buckwheat spaghetti.

Buckwheat tissues can serve as very useful resources for high-quality flavonoids, though flavonoid content varies with development and is significantly influenced by the contents of phenylalanine and tyrosine and the activity of kinetin in the tissues along with different existing forms of nitrogen in the soil (89). The flavonoids in buckwheat seeds depends on variety and cultivating conditions according with the previous study (90). The flavonoid content and composition in seeds vary between different buckwheat species and development phases.

Table 5 showed ALMNO13 flavonoids contents of 24.80 ± 0.89 mg of quercetin/ 100g, a greater amount of total flavonoids than Tartary buckwheat, *F. esculentum* and Common buckwheat, 19.02mg/g, 10 mg/g and 0.28mg/g respectively (92). In comparison *F. tataricum* seeds has higher amount 40 mg/g than the ALMNO 13 variety (89). Between the Buckwheat studied from several researchers the flavonoids content of Tartary buckwheat was larger than common buckwheat. Some authors have reported maximum values of 39.17 mg/100 g in Tartary buckwheat and 56.22 mg/ 100g for common buckwheat (93). The flavonoids in buckwheat seeds depends on variety and cultivating conditions according with the previous study (90; 81). Due to higher content of flavonoids determined in ALMNO13 this new variety can be more effective in antioxidant activity.

Two methods were used to test the antioxidant activity: the DPPH assay, to determine the free radical scavenging activity; and the NO test, to determine scavenging activity against nitric oxide. The DPPH scavenging activity of the ALMNO13 expressed in terms of IC₅₀, was 1.29 mg/mL (Figure 1). These results suggest that ALMNO13 extracts have a high antioxidant. These values are within the range obtained by Velioglu., et al., 1998, in the study of the effect of different solvents on the antioxidant activity of pseudocereals extracts (In regard to the % NO Inhibition are higher than the obtained by Holasova et al., 2002. (69; 94;95).

In general, it is observed that the higher the concentration of antioxidant compounds, higher is the antioxidant activity. The linear regression analysis carried out to establish the correlation between total phenols and the antioxidant capacity (Figure 2), showed a better correlation between total phenols and % NO ($R^2 = 0.6907$) (c) than between total phenols and % DPPH ($R^2 = 0.5678$) (a). When establishing the correlation between flavonoids and the antioxidant capacity, a better correlation was observed between flavonoids and % NO ($R^2 = 0.6849$) (d) than between flavonoids and % DPPH (0.0839) (b).

Table 6. % DPPH Inhibition and % NO Inhibition of new line ALMNO13 buckwheat seeds

Determination	
% DPPH inhibition	83.70 ± 1.86^a
% NO inhibition	48.02 ± 0.78^a

The results are expressed as mean $\pm$ standard deviation of determinations by triplicate. Different letters indicate significant differences ($P < 0.05$) using Student's test.

These results correspond to ethanol extracts with a concentration of 2.5 mg/mL

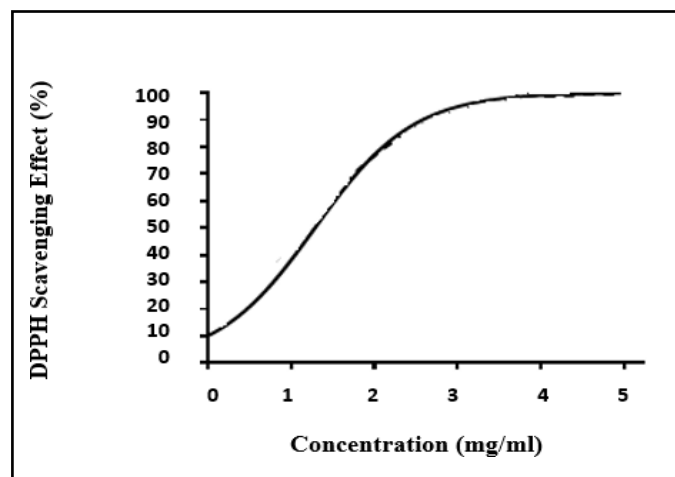


Figure 1. DPPH free radical scavenging activity of. Data are present ALMNO13 buckwheat seeds as percentage of inhibition relative to control. Data are mean $\pm$ SD) (n = 3).

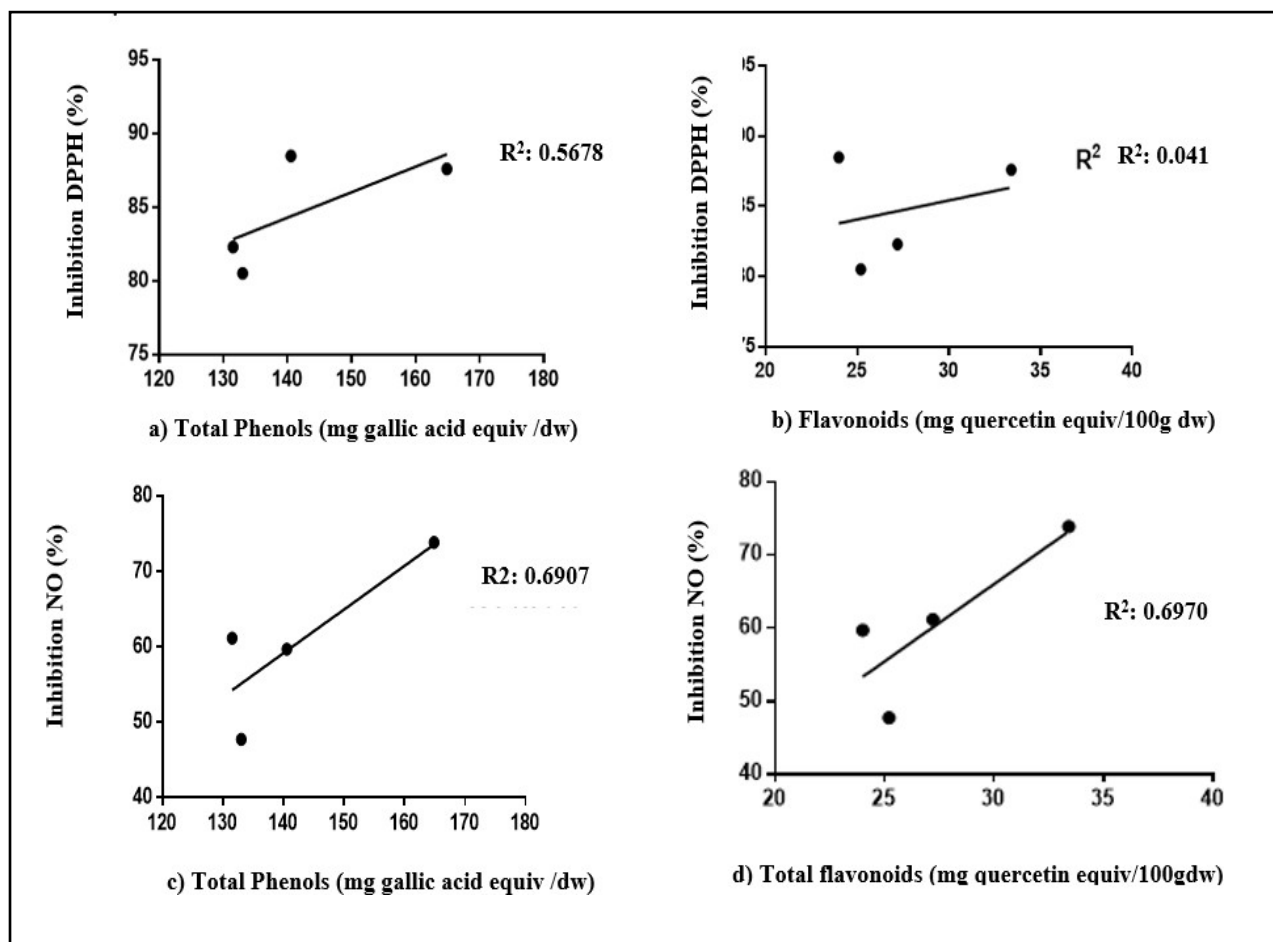


Figure 2. Correlations between the antioxidant activity and total polyphenols and flavonoids contents in ALMNO13 buckwheat seeds. Values correspond to the mean of three determinations $\pm$ SD

These results establish that both, phenols as well as flavonoids, would be actively participating in the sweeping of nitric oxide, but in this case, the flavonoids wouldn't be the DPPH inhibitors, which suggests that this Buckwheat have other bioactive compounds that provoke the inhibition of DPPH. The variety of buckwheat seed act as important dietary antioxidant, and it might be an alternative to replace synthetic antioxidants as additives in food, pharmaceutical and cosmetic preparations.

Lin et al., 2009, proved antioxidant activity by reducing power and DPPH radical scavenging ability in buckwheat enhanced wheat bread. Similarly, Sedej et al., 2011 proved that buckwheat flours exhibited significantly higher ($P < 0.05$) antiradical activity on hydroxyl (OH), superoxide anion (O_2^-) and DPPH radicals, antioxidant activity and reducing power in comparison with wheat fractions. (78;96).

IV. CONCLUSION

The integral use of the buckwheat grain and give the diversification of the market, the global study of its composition is essential. The good results obtained in the evaluation of the nutritional chemical composition, the antinutritional factors are within the allowed values, without risks for human health, and interesting source of antioxidant compounds with a suitable activity, which is expected to have beneficial effects on health of a new buckwheat grain showed an interesting nutritional profile.

In conclusion, proper utilization of this new buckwheat ALMNO13 can be used as part of a daily diet with a great potential in functional food, as an important dietary antioxidant, and could be an alternative to replace synthetic antioxidants as food additives, in addition to contributing to the national food composition database.

We hope our results will provide a starting point for investigations to exploit new natural antioxidants substances present in this and other related species and also promote food industries in the development of new foods.

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