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Nutritional Applications of Quinoa Seeds (Chenopodium quinoa W.) and their Effect on Diabetic Rats

Hanan, M. A. AL-Sayed[1, 2], Nahla, S. Zidan[1, 3], M. A. Abdelaleem[4]*

[1] Department of Nutrition and Food Science, Faculty of Home Economics, Tabuk University, Saudi Arabia.
[2]Food Science department, Faculty of Agricultural, Ain Shams University, Cairo, Egypt.
[3]Department of Home Economics, Faculty of Specific Education, Kafr ElShaikh University, Egypt.
[4]Plant Research Department, Nuclear Research Center, Atomic Energy Authority, Cairo, Egypt.

***Email:** Mohamed.Abdelrazek@eaea.org.eg*

ABSTRACT

The aim of this work was to evaluate the chemical composition; mineral and vitamin contents; total phenolic and flavonoids contents; antioxidant activity of the quinoa seeds powder (QSP) and their effect on diabetic rats. The higher concentrations of minerals were K (3441.95 mg/Kg) and Mg (1147.32 mg/Kg) and quinoa seeds contained a considerable amount of riboflavin (B2), pyridoxine (B6), folic acid (B9), cobalamin (B12), alpha tocopherol (E) and beta carotene equal to 0.60, 5.83, 6.80, 0.27, 2.010 and 0.127 mg/100g, respectively. The total phenolic and total flavonoids content of quinoa seeds powder were 2.63 mg GAE/g, and 0.53 mg CE/g, respectively. Antioxidant activity was measured as 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH) and 2,2´-azinobis (3-ethyl benzo thiazoline-6-sulphonic acid) radical cation (ABTS) were 0.29 and 4.38 mg TE/g, respectively. Sensory properties of both quinoa pudding and quinoa soup showed insignificant differences. However, in biological experiment, the blood and the glucose levels after consumption of quinoa at different concentrations were reduced from 236.7 mg/dl to 98.7mg/dl at 40% quinoa and to 120.3 mg/dl at 30% quinoa with an average fasting of normal level of 64.3 mg/dl. Furthermore, the thyroid hormones T3 and T4 were reduced significantly after feeding rats on quinoa seeds powder at different concentrations. Histopathological determination of pancreas of diabetic rats revealed that interlobular inflammatory cells infiltration where pancreas of diabetic rats fed on 20, 30 and 40% quinoa powder revealed no histopathological changes.

Key words: *Quinoa, Phenolic, Antioxidant activity, Diabetic, Histopathology, Applications.*

INTRODUCTION

Diabetes is a common disease worldwide in which the concentration of blood glucose is chronically raised. It is occurred due to the lack of insulin either absolutely or relatively, insulin is not being produced from the pancreas or there is insufficient insulin or insulin action for the body's needs. [1, 2]

Hyperglycemia can make oxidative stress that may result in cellular tissue damage. Furthermore, it can have a harmful effect on the metabolism of tissues and organs. Hyperglycemia leads to malfunction of organs if it remains uncontrolled. [3] Quinoa is considered as a pseudo-cereal, it is not a member of the *Gramineae* family and it produces seeds that can be milled into flour and used as a cereal crop. Seed's color is usually pale yellow but may have other different colors like pink, orange, red, brown and black. [4]

Quinoa is crop grown mainly as a cereal food. Concerning the most important nutrients, it is considered as the world's one of the most popular health foods. Quinoa has high nutritive value and can achieve the food security worldwide. 2013 was considered as "the international year of the quinoa". [5]

Quinoa that contains a complete protein, has high amount of essential amino acids and fatty acids and it is a good source of vitamin C, E and several B vitamins. [6] Quinoa is considered as a grain substituted in gluten free diets and most people get the majority of their B vitamins from baked goods. Like milk protein, quinoa protein content is ranged between 14 and 18%. Quinoa is also a source of calcium, magnesium, zinc and iron. [7] Quinoa seeds have an excellent nutrient characterization; furthermore, they are a good and main source of energy because their contain starch, lipids (un-saturated fats), dietary fiber and good-quality protein. [7, 8] Several studies mentioned that quinoa seeds contain high amount of phytochemicals that play an important role as antioxidants *i.e.;* phenolic acids, flavonoids, fat soluble vitamins, trace elements, fatty acids, and squelene. [9-11]

Most low income countries and communities suffer from diabetes which is a metabolic disorder that has reached epidemic proportions in such countries. [12] Glycemic index is a measure of how quickly foods raise blood sugar levels. Using a scale of 0-100, glycemic scores are separated into three groups- low, moderate and high. Any food with a score of 55 or less falls in the low glycemic range, 56-69 in moderate and 70 or above in high glycemic category. Low glycemic index food improves glucose and lipid levels and weight control. In addition, they reduce insulin resistance and risk of cardiovascular diseases, diabetes and some cancers. Insulin resistance and the risk of cardiovascular diseases, diabetes and some cancers can be reduced using low GI diets. [13-15] Quinoa seeds have been used as foods in different ways, like whole grains, sauces, porridges, tasteful soups, sweets, beverages, and soufflés, starchy material, and protein concentrates. In addition, seeds can be ground and used as a flour ingredient in various mixtures for breads, cakes, pancakes, crackers, muffins, dumplings, cookies, pasta and puddings. [16, 17]

As a protein supplement in wheat flour and also in preparation of cakes, pastries, pasta and baked goods, the highly nutritive quinoa flour is usually used. [18]. Currently, there have been advances in research on the use of quinoa flour in composite flour for making bread products, biscuits and pastries. Gluten-free quinoa is characterized by high levels of protein with all essential amino acids, vitamins, unsaturated fatty acids and minerals as well as, low glycemic index. [19, 20]

Quinoa has no gluten. So, it is an interesting ingredient for the diets of celiac disease persons. Quinoa flour is used also in infant foods. Flakes were also prepared from quinoa. [4] As quinoa is easy to cook, it is easy to prepare instantly and could be cultivated in different environments. So, quinoa is known as a food of low social prestige and it attracts the attention of many countries worldwide. [5]

Thus, the aim of our study was to evaluate the biochemical, and histopathological changes that occur in diabetic albino rats fed on different concentrations of quinoa seeds powder. In addition, determination of the nutritional properties, chemical composition, phenolic content, and antioxidant activity in quinoa seeds powder and determination of the sensory properties of both quinoa pudding and quinoa soup as nutritional applications are among the objectives of the study.

MATERIALS AND METHODS

Materials

Plant materials

Quinoa seeds (*Chenopodium quinoa*) was obtained from the local market, Tabuk, Saudi Arabia. The quinoa seeds were washed, dried and crushed using electric blender to obtain a fine powder.

Pudding and soup ingredients

Whole milk, vanilla, starch and lentil seeds were purchased from local market, Tabuk, Saudi Arabia.

Chemicals

All chemical reagents were obtained from Sigma (St. Louis, MO), unless otherwise specified. All solvents used for compound isolation were HPLC grade. 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (TROLOX) and 2,2´-azino-bis (3-ethyl benzo thiazoline-6-sulphonic acid (ABTS) were purchased from Sigma-Aldrich, Germany.

Chemical composition of quinoa seeds powder

Chemical composition of quinoa seeds powder including the contents of moisture, ash, crude protein and crude fat were determined according to A.O.A.C (2007). [21] Carbohydrates were determined by difference.

Determination of minerals

Iron, calcium, potassium and magnesium were determined in quinoa seeds powder using atomic absorption spectrum. [21]

Water-Soluble Vitamins

Ten grams of quinoa seeds powder was weighed, homogenized in mortar with pestle, transferred into conical flask and 25 mL of the extraction solution (made by mixing 50 mL of acetonitrile with 10 mL of glacial acetic acid and the volume was finally made up to 1000 mL) was added. The prepared solution of quinoa seeds powder was injected into HPLC by using auto-sampler to determine water soluble vitamins. [22]

Tocopherols and β-carotene

Tocopherols and β-carotene were determined in quinoa seeds powder according to Annunziata *et al*. (2012). [23]

Determination of Antioxidant Activity

Extraction

Extracts for total phenolic, total flavonoids and antioxidant activity were prepared using methanol. One gram from quinoa seeds powder (QSP) was mixed with 100 mL methanol and homogenized using the Ultra-Turrax homogenizer. The homogenates were kept at 4 °C for 12 h and then centrifuged at 10,000 rpm for 20 min. The supernatants were recovered and stored at -20 °C until analysis.

Determination of total phenolic content (TPC)

The total phenolic content of quinoa seeds powder (QSP) was determined according to Folin-Ciocalteu procedure. [24] A calibration curve which was prepared with gallic acid was used to determine the total phenolic content, and expressed as milligrams of Gallic Acid Equivalent (GAE) per g of sample. In case the absorbance value measured was over the linear range of the standard curve, additional dilution was done.

Determination of total flavonoid content (TFC)

Total flavonoid content (TFC) of the extract of quinoa seeds powder (QSP) was determined using aluminium chloride ($AlCl_3$) method according to a reliable approach using quercetin as the standard. [25] The results were expressed as milligrams of Cateachin Equivalent (CE) per g of dry material.

Determination of radical DPPH scavenging activity

Free radical scavenging capacity of quinoa seeds powder extracts (QSPE) was determined using the stable DPPH• according to Hwang and Do Thi (2014). [26] The final concentration was 200 μM for DPPH• and the final reaction volume was 3.0 mL. After 60 min of incubation in a dark condition, the absorbance was measured at 517 nm against a blank of pure methanol. Using the following equation, percent inhibition of the DPPH free radical was calculated:

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

Where: $A_{control}$ is the absorbance of the control reaction which contains all reagents except the test compound. A_{sample} is the absorbance with the test compound. The standard curve was prepared using Trolox. The results were expressed as mg Trolox equivalents (TE / g sample). Additional dilution was needed if the DPPH value measured was over the linear range of the standard.

Determination of radical ABTS scavenging activity

The stock solutions of ABTS• reagent was prepared according to Hwang and Do Thi (2014) by reacting equal quantities of a 7 mM aqueous solution of ABTS• with 2.45 mM potassium persulfate for 16 h at room temperature (25°C) in the dark. Then, by diluting 1 mL ABTS• solution with 60 mL of ethanol: water (50:50, v/v), the working solution was prepared to obtain an absorbance of 1.0± 0.02 units at 734 nm using the spectrophotometer. In a dark condition, the extracts (50 μL) were allowed to react with 4.95 mL of the ABTS• solution for 1 h. Then, using the spectrophotometer, the absorbance was taken at 734 nm. Percent inhibition of the ABTS• free radical was calculated by the following equation:

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

Where: $A_{control}$ is the absorbance of the control reaction which contains all reagents except the test compound. A_{sample} is the absorbance with the test compound. The standard curve was prepared using Trolox. The results were expressed as mg Trolox equivalents (TE / g sample). Additional dilution was needed if the ABTS• value measured was over the linear range of the standard.

Biological experiment

Experimental Design

A total of 55 male albino rats, average weight of (170-180 g) were used in the present study in the animal lab of the Research Institute of Ophthalmology, Giza, Egypt. All rats were housed in individual cages in an air-conditioned room where the temperature of 25 ± 1 ºC and 12 h light and dark cycle were maintained for a period

of 6 weeks with free access to food and water. The rats were divided into sex groups and treated as follows: Group (I): a normal control group; Group (II): a diabetic control group; Group(III): a diabetic group treated with 10% QSP (IV): a diabetic group treated with 20% QSP; Group (V): a diabetic group treated with 30% QSP and Group (VI): a diabetic group treated with 40% QSP. The guide for the care and use of laboratory animals was used as the standard which was adopted and promulgated by the Institutional Animal Care Committee.

Induction of Diabetes in Rats

Diabetic groups (II) to (VI) were induced by a single intravenous injection of 120 mg/kg body weight alloxan monohydrate (Sigma chemicals, USA) [27] dissolved in 0.1 M citrate buffer (pH 4.00). The alloxan-treated rats were given 5% glucose water for 24 h following alloxan injection, to prevent initial drug induced hypoglycemic mortality. After 7 days of alloxan injection, the tail vein blood was collected to determine fasting blood glucose (FBG) level. [28] Only the rats with FBG over 250 mg/dl were considered diabetic and distributed into groups (II) to (VI) in the experiments. The exclusion criterion included the rats that did not reveal any FBG levels increase even after alloxan injection; accordingly, considered as totally resistant. The rats were housed in individual cages in an air conditioned room (25 ± 1 ^{o}C) and 12 h light and dark cycle was maintained. The composition of basal standard diet was prepared according to Reeves *et al*. (1993). [29]

Blood sampling

Blood samples were taken at the end of the experiment (42 days) of the administration of the powder of GCS. The blood samples were obtained from orbital plexus venous (under diethyl ether anesthesia) as mentioned by Dhandapani *et al*. (2002), [30] by means of the fine capillary glass tubes based on Schermer method (1967). [31] Each sample was placed in a dry and clean centrifuge tube and allowed to clot (undisturbed) for 1-2 hr. at 37 ^{o}C. Sera was then removed using a Pasteur pipette and centrifuge for 10 min. at 3000 rpm to remove any suspended red blood cells. The clean non haemolysed supernatant serum was then pipetted into a Wasserman tube and kept frozen at -17 ^{o}C until analysis for blood glucose and thyroid hormones.

Biochemical analysis

Determination of glucose

Glucose in serum was determined by enzymatic colorimetric method according to Barham and Trinder (1972).

Determination of thyroid hormones thyroxine (T3) and triiodothyronine (T4)

Serum T3 and T4 were measured using conventional radioimmunoassay (RIA) kits. (T3: Cat. No. MG13081 and T4: Cat. No. MG13091) IBL, Hamburg, Germany.

Histopathology

In different groups of rats, autopsy samples were taken from pancreas and then fixed in 10% formal saline for 24-hour period. Washing process was carried out through using tap water and then for dehydration, serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used. In the condition of 56-degree hot air oven for 24-hour period, the specimens were cleared in xylene embedded in paraffin. For sectioning at 4 microns thickness by slide microtome Paraffin bees wax tissue blocks were prepared. Eventually, the obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin stains aimed at histopathological examinations through the light microscope. [32]

Nutritional applications of Quinoa seeds

Preparing quinoa seeds

Quinoa seeds were soaked at room temperature four 1h and blanched for 15 min, and then cooled at room temperature.

Quinoa pudding

Quinoa pudding was prepared by mixing the 50 ml milk, 4.2 g starch, 2.5 g vanilla and water up to 100 ml with blanched quinoa at different concentrations (0, 10, 20, 30, 40 and 50%) and the dispersion was heated at 90°C for 6 min under strong agitation. The pudding samples were placed in closed glass containers, cooled to room temperature (25°C) and then stored in a refrigerator (4 - 5°C) for 24h (Fig. 1). [33]

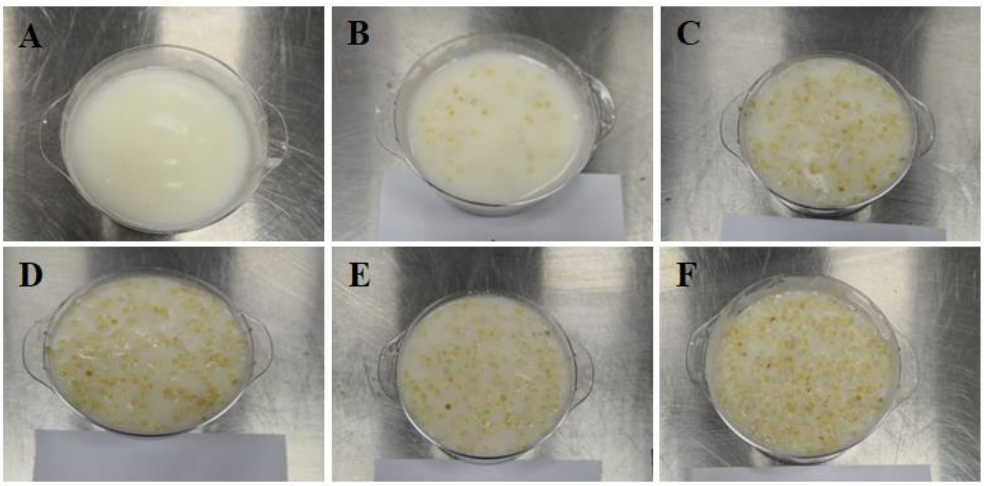

Figure 1. Quinoa pudding containing different concentrations; A) 0%, B) 10%, C) 20%, D) 30%, E) 40%, F) 50% of quinoa seeds.

Quinoa soup

Quinoa soup was prepared by mixing blanched quinoa, blanched lentil and water in blender at the following concentrations; control quinoa (100 g quinoa/ 100 ml water), control lentil (100 g lentil/ 100 ml water), 1 : 1 ratio (50g quinoa and 50g lentil / 100 ml water), 3 : 1 ratio (75g quinoa and 25g lentil / 100 ml water), 1 : 3 ratio (25g quinoa and 75g lentil / 100 ml water) (Fig. 2).

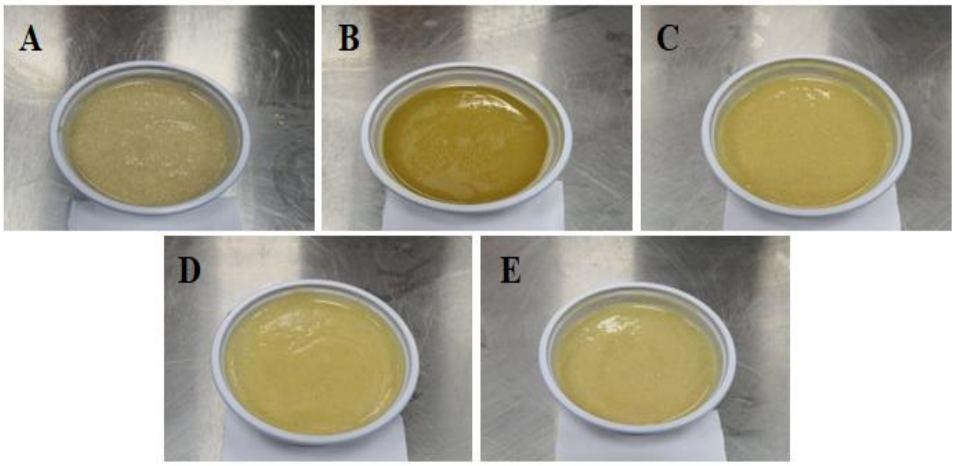

Figure 2. Quinoa and lentil soup at different concentrations; A) 100% quinoa, B) 100% lentil, C) 50% quinoa: 50% lentil, D) 75% quinoa: 25% lentil, E) 25% quinoa : 75% lentil.

Sensory evaluation

Ten panelists from the staff members of nutrition and Food Science Department, Faculty of Home Economics, Tabuk University were asked to evaluate texture, taste, color, odor and overall acceptability of the processed quinoa pudding and quinoa soup according to Ares *et al*. (2009). [33]

Statistical analysis

The experimental data were analyzed using analysis of variance and Duncan multiple range test at ($p < 0.05$). The data were analyzed according to User Guide of Statistical Analysis System.

RESULTS AND DISCUSSION

Chemical composition of quinoa seeds powder

Nowadays, consumers who are health-conscious, are figuring out a preference for value-added products. Complete or supplement replace of common cereals (rice, wheat and corn) with nutritional cereals (like quinoa) is inherently beneficial to the public interest. [34]

As shown in Table (1), the chemical composition of the quinoa seeds powder on dry weight basis was illustrated. The present results showed that quinoa seeds powder had content of moisture, protein, fat, ash, fiber and total carbohydrates, as follows (11.78 %, 12.91%, 4.06%, 2.50%, 5.14% and 63.51%, respectively). As mentioned by Vega-Galvez *et al*. (2010), the quinoa has higher content of vegetable protein source than found in wheat, rice, barley, rye, corn, sorghum and maize. [35]

The present results are in agreement with those of Kozioł (1992), [36] who found that oil content in quinoa ranges from 1.8% to 9.5%, with an average of 5.0 – 7.2%, which is higher than that of maize (3 – 4%) and our results are in line with Lamothe *et al*. (2015), [15] who found that quinoa is perfect source of dietary fiber, it has about 2.6% - 10% of the total weight of the grain. Maki and Phillips (2015) reported that with the greater consumption of grains with higher fiber concentration, the lower risk of type 2 diabetes is appeared. [14]

Some minerals content of quinoa seeds powder

Dietary minerals are essential chemical elements for Electrolyte balance. Glucose homeostasis is mainly used as enzyme cofactors as well as nerve impulses in the body. [37] The same mineral content of quinoa powder is presented in Table (1). In the study, quinoa seeds powder contain high amount of ash (2.50%) that is closed with Vega-Galvez *et al*. (2010) and Repo-Carrasco *et al*. (2003) who explained that quinoa's ash content (3.4%) is higher compared to rice (0.5%), wheat (1.8%), etc.; [35, 38] accordingly, large amounts of minerals are found in quinoa seeds. The present results in Table (1) showed that quinoa seeds powder had content of potassium, magnesium, calcium and iron as follows (3441.95, 1147.32, 430.24 and 28.68 mg/Kg, respectively). Quinoa has a biological forms of magnesium, calcium and potassium, these minerals are considered to make sufficient balanced diet. [35, 38] Also, Ruales and Nair (1993) mentioned that quinoa seeds play as rich source of minerals (iron, magnesium, manganese and phosphorus) compared to most of common grains. [39]

Table 1. The chemical composition and minerals content of quinoa seeds powder

		Quinoa seeds powder
Chemical composition (%)	**Moisture**	11.78
	Protein	12.91
	Fat	4.06
	Ash	2.50
	Fiber	5.14
	Carbohydrates*	63.51
Minerals (mg/Kg)	**K**	3441.95
	Mg	1147.32
	Ca	430.24
	Fe	28.68

* Total carbohydrate was calculated by difference

Total phenolic, flavonoid content and antioxidants activity of quinoa seeds powder

Like most of plants, quinoa has phenolic compounds which play an important role as bioactive phytochemicals. [40] Because of health promoting properties of phenolic compounds, polyphenols recently have been further researched. These compounds inhibit both free radicals and oxidative chain reaction. [41, 42]

The total phenolic, flavonoid content and antioxidants activity of the quinoa seeds powder are illustrated in Table (2). The present results showed that quinoa seeds powder had total phenolic (2.63 mg GAE/g), Total flavonoids (0.53 mg CE/g) and antioxidants activity as follows DPPH (0.29 mg TE/g) and ABTS (4.38 mg TE/g). Gorinstein *et al*. (2007) observed that in quinoa seeds a relatively high concentration of total phenolics 3.75 mg gallic acid equivalents per g. [9] The results given in Table (2) indicated that total phenolics (2.63 mg GAE/g) was relatively low because of differences in extraction methodology as illustrated by Navruz and Sanlier (2016) in plant system, the antioxidant activity of chemical constituents depends mainly on genotype, growing conditions, seasons, maturity, post-harvest and storage. [43]

Table 2. Total phenolic, flavonoid content and antioxidant activity of quinoa seeds powder

	Concentration
Total phenolic Content (TPC)	2.63 mg GAE/g

Total flavonoids Content (TFC)	0.53 mg CE/g
DPPH	0.29 mg TE/g
ABTS	4.38 mg TE/g

GAE = Galic Acid Equivalent; CE = Cateachin Equivalent; TE = Trolox Equivalent

Phenolic profile

The results given in Table (3) indicate that quinoa seeds contained considerable amount of phenolic compounds with an average from 3059.2 to 9.3 (µg/100g). Table (3) shows phenolic profile of quinoa seeds powder by HPLC which are Protocatechuic, Kaempferol, Caffeic, Syringic , Vanillic, ferulic, Chrysin, sinapic, *p*-coumaric Apigenin-7-glucoside, Rosmarinic and cinnamic equal to 21.2, 13.6, 49, 22.8, 285.9, 3059.2, 9.3, 244.9, 65.8, 52.1, 14.6, 342.1, 107.7 and 44.1 (µg/100g), respectively. It could be observed that ferulic and rosmarinic are present in the highest levels comparing with other phenolic compounds present in moderates concentrations such as vanillic and sinapic. While data in the same Table revealed that chrysin and kaempferol are present in the lowest levels.

Table 3. Phenolic profile of quinoa seeds powder

Phenolic profile	**Concentration (µg/ 100g)**
Protocatechuic	21.2
Cateachin	ND*
Kaempferol	13.6
Caffeic	49
Syringic	22.8
Vanillic	285.9
Ferulic	3059.2
Chlorogenic	ND*
Chrysin	9.3
Sinapic	244.9
p-coumaric	65.8
Rutin	52.1
Apigenin-7-glucoside	14.6
Rosmarinic	342.1
Cinnamic	107.7
Apigenin	44.1

*ND = Not Detected,

Main vitamins content of quinoa seeds powder

Vitamins are compounds essential for the health of humans and animals. Traditionally, vitamins have been among the most widely applied chemical agents to enhance the nutritional values of food products. [44] Quinoa also contains significant amount of vitamin E, which acts as an antioxidant, although the quantity declines after processing and cooking. [45]

The results given in Table (4) indicates that quinoa seeds contain considerable amount of Riboflavin (B2), Pyridoxine (B6), Folic acid (B9), Cobalamin (B12), Alpha Tocopherol (E) and Beta carotene as follows (0.60, 5.83, 6.80, 0.27, 2.010 and 0.127 mg/100g, respectively).

Quinoa contains high concentrations of various B vitamins like pyridoxine (B6) and folic acid (B9). The adults' daily needs form quinoa of both vitamins were 100 g. [46] On the other hand, Alvarez-Jubete *et al*. (2010) reported that the levels of vitamins pyridoxine, folic acid and riboflavin in quinoa are higher compared to other grains like oat, corn wheat, barely, rice and rye. [47] Furthermore, quinoa has the highest amount of vitamin E in pseudo-cereal.

Table 4. Vitamins content, Tocopherol and Beta carotene of quinoa seeds powder

	Total Content (mg / 100 g)
Riboflavin (B2)	0.60
Pyridoxine (B6)	5.83
Folic acid (B9)	6.80
Cobalamin (B12)	0.27
Alpha Tocopherol (E)	2.010
Beta carotene	0.127

Nutritional applications of Quinoa seeds

Quinoa Pudding

The mean values of sensory properties of pudding containing different concentrations of quinoa seeds are given in Table (5). Quinoa pudding samples containing different concentrations of quinoa seeds showed to be significantly superior in color, taste, texture, odor, and overall acceptability. Statistical analysis of panelist scores for sensory properties of quinoa pudding containing different concentrations of quinoa seeds were evaluated to choose their best concentrations. It was noticed that the addition of quinoa seeds to pudding recorded to be the highest at concentration of 30, 40, and 50% followed by 20 and then 10%. The increasing of added concentration (from 20 to 50 %) of quinoa seeds increased all sensory properties of quinoa pudding samples.

Quinoa Soup

The mean values of sensory properties of quinoa soup containing different ratios of quinoa and lentil seeds are given in Table (6). Quinoa soup samples containing (1 quinoa: 3 lentil) showed to be significantly superior in color, taste, texture, odor, and overall acceptability. Statistical analysis of panelist scores for sensory properties of quinoa soup containing different ratios of quinoa and lentil seeds were evaluated to choose their best ratio and it was noticed that the soup samples recorded the highest at ratio (1 quinoa : 3 lentil) followed by (3 quinoa :1 lentil) compared to the control soup (quinoa) and control soup (lentil).

Table 5. The sensory properties of quinoa pudding containing different concentrations of quinoa seeds powder

Concentration of quinoa seeds powder (%)	**Sensory properties**				
	Color	**Taste**	**Texture**	**Odor**	**Overall acceptability**
0	$9.0^{a} \pm 1.2$	$9.0^{a} \pm 0.0$	$9.4^{a} \pm 0.7$	$9.6^{a} \pm 0.3$	$9.7^{a} \pm 0.6$
10	$7.6^{a} \pm 0.5$	$9.0^{a} \pm 0.0$	$7.6^{a} \pm 0.5$	$8.6^{a} \pm 0.4$	$8.1^{a} \pm 0.4$
20	$8.3^{a} \pm 0.5$	$9.0^{a} \pm 0.0$	$8.4^{a} \pm 0.2$	$9.1^{a} \pm 0.2$	$8.6^{a} \pm 0.1$
30	$8.5^{a} \pm 0.5$	$9.0^{a} \pm 0.0$	$8.5^{a} \pm 0.5$	$9.3^{a} \pm 0.2$	$9.1^{a} \pm 0.3$
40	$8.8^{a} \pm 0.6$	$9.0^{a} \pm 0.0$	$8.8^{a} \pm 0.6$	$9.4^{a} \pm 0.3$	$9.3^{a} \pm 0.7$
50	$8.9^{a} \pm 0.7$	$9.0^{a} \pm 0.0$	$8.9^{a} \pm 0.8$	$9.5^{a} \pm 0.5$	$9.3^{a} \pm 0.6$

Means having different letters (superscript) in the same column are significantly different ($P < 0.05$).

Table 6. The sensory properties of quinoa Soup containing different ratios of quinoa and lentil seeds

	Sensory properties				
	Color	**Taste**	**Texture**	**Odor**	**Overall acceptability**
Control (quinoa)	$7.6^{a} \pm 0.6$	$9.8^{a} \pm 0.0$	$7.7^{a} \pm 1.0$	$7.4^{a} \pm 1.1$	$7.8^{a} \pm 0.9$
Control (lentil)	$7.8^{a} \pm 0.8$	$9.8^{a} \pm 0.0$	$7.8^{a} \pm 0.8$	$8.1^{a} \pm 1.1$	$7.8^{a} \pm 0.9$
1 quinoa: 1 lentil	$8.1^{a} \pm 0.3$	$9.8^{a} \pm 0.0$	$8.3^{a} \pm 0.3$	$8.1^{a} \pm 0.2$	$8.4^{a} \pm 0.3$
3 quinoa: 1 lentil	$8.5^{a} \pm 0.4$	$9.8^{a} \pm 0.0$	$8.8^{a} \pm 0.4$	$8.6^{a} \pm 0.4$	$8.6^{a} \pm 0.5$
1 quinoa: 3 lentil	$8.8^{a} \pm 1.1$	$9.8^{a} \pm 1.0$	$9.1^{a} \pm 1.2$	$9.0^{a} \pm 1.1$	$9.1^{a} \pm 1.2$

Means having different letters (superscript) in the same column are significantly different ($P < 0.05$).

Biological analysis

Quinoa has attracted attention in the last years not only for its excellent nutritional value, but also for containing high amounts of proteins, fibers, minerals, vitamins and ω -3 fatty acids. [35]

Table 7. Effect of feeding with experimental diet supplemented with different concentrations of quinoa powder on body weight gain

	Initial weigh	Final weigh	Net gain	Daily	%
Normal Rats fed on Basel diet					
Normal rats	$168.75^{a} \pm 2.39$	$222.50^{ab} \pm 9.2$	$53.75^{a} \pm 9.6$	$1.792^{a} \pm 0.32$	$179.2^{a} \pm 32.2$
Diabetic rats	$172.50^{ab} \pm 3.20$	$200.00^{a} \pm 0.0$	$27.50^{a} \pm 3.2$	$0.917^{a} \pm 0.11$	$91.67^{a} \pm 10.7$
Diabetic rats fed on quinoa powder					
10% Quinoa	$173.75^{b} \pm 2.39$	$231.25^{b} \pm 11.4$	$57.50^{a} \pm 11.6$	$1.917^{a} \pm 0.38$	$191.7^{a} \pm 38.8$
20% Quinoa	$175.00^{ab} \pm 0.00$	$226.25^{ab} \pm 10.3$	$51.25^{a} \pm 10.3$	$1.708^{a} \pm 0.34$	$170.8^{a} \pm 34.3$
30% Quinoa	$177.50^{ab} \pm 3.20$	$225.00^{ab} \pm 8.7$	$47.50^{a} \pm 10.1$	$1.583^{a} \pm 0.33$	$158.3^{a} \pm 33.7$
40% Quinoa	$173.75^{b} \pm 2.39$	$221.25^{ab} \pm 7.7$	$47.50^{a} \pm 6.6$	$1.583^{a} \pm 0.22$	$158.3^{a} \pm 22.0$

Means having different letters (superscript) in the same column are significantly different ($P < 0.05$).

Table (7) summarizes the body weight gain (%). Non-significant differences ($P > 0.05$) were indicated in net gain in all treated groups compared with the control group. Daily gain in body weight also followed the same trend where it observed non-significant differences between the treated groups and control group. Several physiological effects on human health of quinoa consumption were investigated in several animal studies. In the mentioned studies, the effects of quinoa consumption included non-significant differences ($P > 0.05$) in weight gain of male Wister rats (albino strain). [48] On the contrary, Gee *et al*. (1993) found that the control group of Wister rats gained more weight than the bitter, washed bitter, and sweet quinoa groups. [49]

Nowadays, quinoa is well known as a well-balanced diet. In addition, it has a reduction effect on the chronic disease risk. Quinoa has been recently used as a source to maintain sugar levels. At the end of experiment (6 weeks), glucose was determined immediately after sacrificing. The glucose value was decreased significantly as shown in the Figure (3) in the diabetic rats that had (10 – 40 %) quinoa powder in the administered diet compared to the diabetic rats fed on a basal diet.

Our data is line with Graf *et al*. (2014) who mentioned that when obese, hyperglycemic mice were given a supplement made by leaching nutrients from quinoa seeds, their fasting blood sugar dropped. [50] Similarly, a quinoa-fortified diet decreased blood sugar levels compared to those without quinoa supplementation. [51]

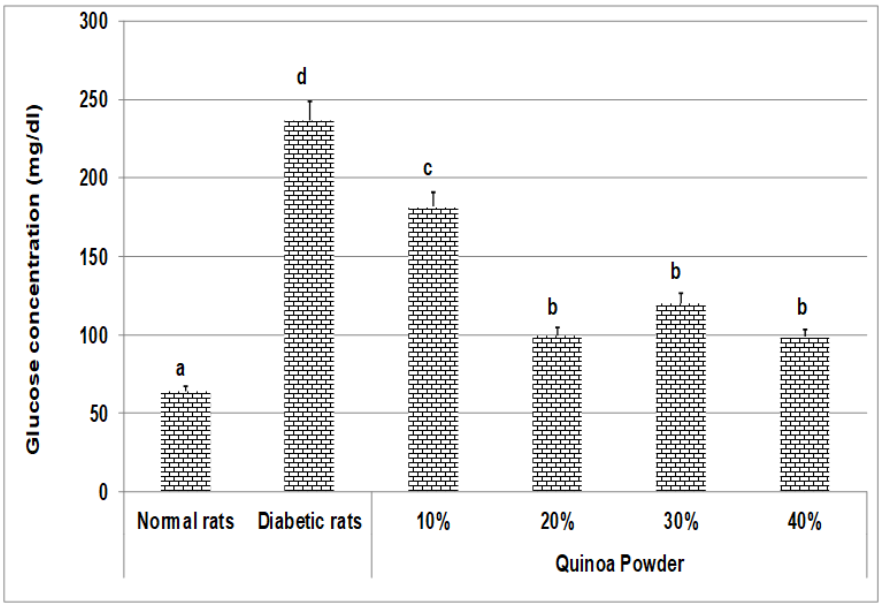

Figure 3. Effect of different concentrations of quinoa powder administration on fasting blood glucose in diabetic rats

Controlling diets is mainly considered as controlling of diabetes, and quinoa is one of these diets as great choice for controlling diabetes. It is one of the various components of a healthy diabetic diet as well as fruits and vegetables, lean proteins and unsaturated fats. Quinoa does have fructose and glucose levels comparatively

lower than starches in other grains. The essential fatty acids in quinoa are the types that have been linked to improve insulin sensitivity (i.e. insulin is better able to get glucose out of the blood stream and into cells. [51]
As mentioned in preceding studies, quinoa as a fiber-rich-source plays an important role that impacts blood sugar besides maintaining body weight to prevent chronic diseases accompanied by diabetes, and fiber has been shown to improve the response of insulin after eating. [35, 52, 53]
Pasko *et al*. (2010) reported that feeding quinoa in a diet to rats on a high-fructose diet, reduced most of the adverse effects caused by fructose, all of which are associated with type 2 diabetes. [54] Valencia-Chamorro (2003) reported 2% mono-saccharides and 2.3% disaccharides in quinoa. [55] Maltose and d-xylose are found in quinoa flour with high percentages while it has a lower concentrations of glucose and fructose which allows its use in malted drink. [56]

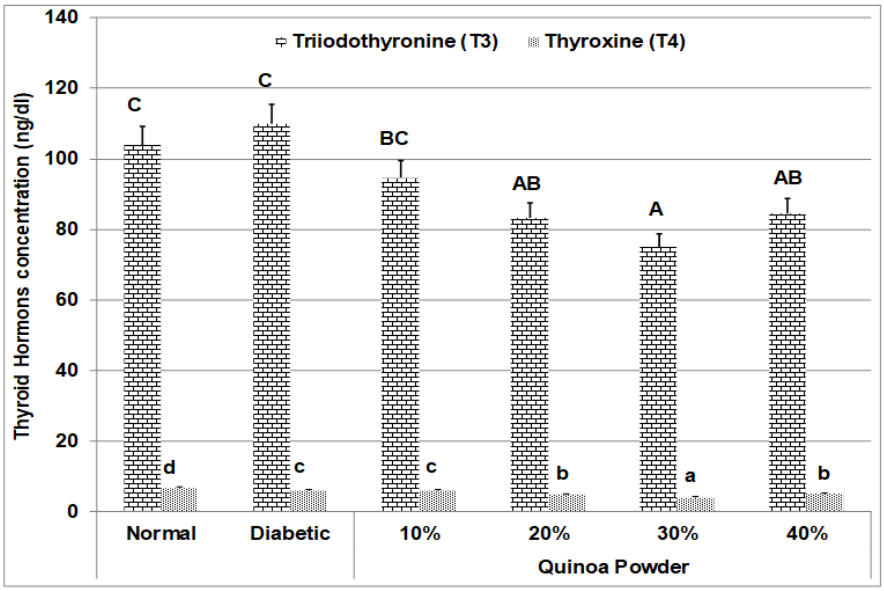

Figure 4. Effect of different concentrations of quinoa powder administration on thyroid hormones in rats

As shown in Figure (4), thyroid hormones thyroxine (T_4) and triiodothyronine (T_3) were significantly ($P > 0.05$) decreased in diabetic rats fed on the different concentrations (20, 30 and 40 %) of quinoa powder compared to diabetic rats fed on Basel diet. On the other hand, concentration 10% quinoa did not show significant differences in thyroxine (T_4). However, the treatment of administration quinoa powder proved that quinoa has caused hypoglycemic effect in diabetic rats leading to T_3 and T_4 hormones decrease.

Histopathological examination results

Pancreas of normal rats fed on Basel diet showed no histopathological changes (Fig. 5A). In addition, pancreas of diabetic rats showed interlobular inflammatory cells infiltration (Fig. 5B). However, pancreas of diabetic rats fed on 10% quinoa powder (Fig. 5C) showed vacuolation of cells of islets of Langerhan's. Finally, pancreas of diabetic rats fed on 20, 30 and 40% quinoa powder, respectively revealed no histopathological changes (Fig. 5D, 5E and 5F).

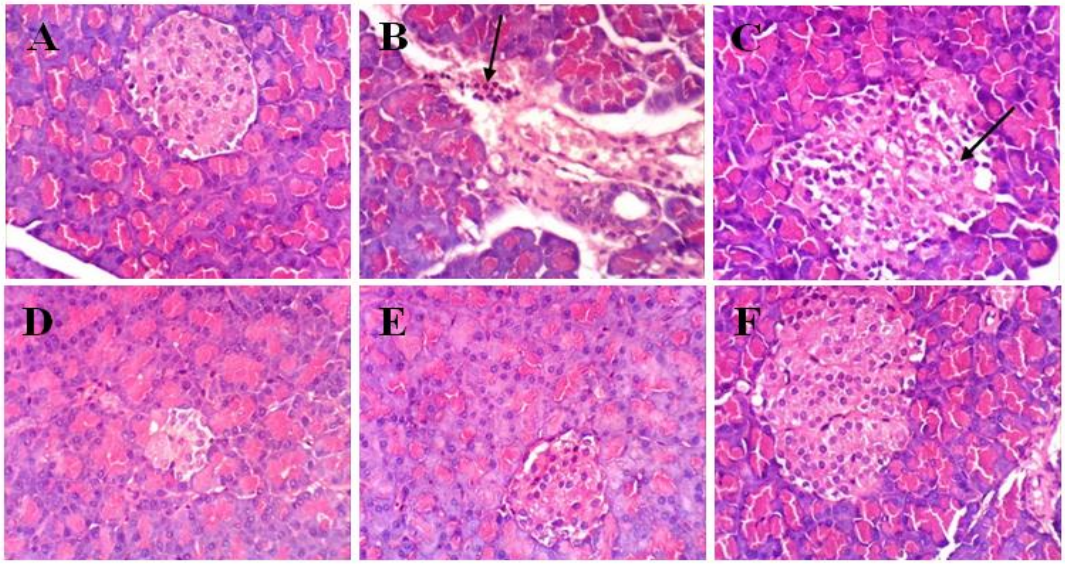

Figure 5. Pancreas of rat from **A)** normal rats showing no histopathological changes (H & E X 400), **B)** diabetic rats showing interlobular inflammatory cells infiltration, **C)** diabetic rats fed on 10% quinoa powder showing vacuolation of cells of islets of Langerhan's, **D)** diabetic rats fed on 20% quinoa powder showing no histopathological changes, **E)** diabetic rats fed on 30% quinoa powder showing no histopathological changes, **F)** diabetic rats fed on 30% quinoa powder showing no histopathological changes.

CONCLUSION

It can be concluded that quinoa may benefit high-risk group consumers, such as diabetes, obesity, children, the elderly, due to its properties including a high nutritional value, and therapeutic features. These characteristics are considered in our work to be correlated with the existence of the fiber, minerals, vitamins, antioxidants, and especially phytochemicals in quinoa seed powder. The blood glucose value was decreased significantly in the diabetic rats that had (10 – 40 %) quinoa powder in the administered diet compared to the diabetic rats fed on a basal diet that they show it big advantage over other crops in terms of human nutrition and health maintenance**.** Quinoa seeds should be recommended for utilization on a commercial scale in the Saudis meals and factories since such seeds have the capability to give more protection against diabetic disease**.**

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