

Effect of Different preparatory processes on Improving the Nutritional Value of Quinoa (*Chenopodium quinoa*, Willd) and Studying its Effect on Obese Anemic Rats

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Abstract

Chenopodium quinoa Willd. is a grain of great nutritional value and has gained importance in most countries. The quinoa seeds go through many processes that may change their nutritional value before consumption. The effect of (germination, soaking, fermentation with or without soaking and cooking under pressure) on chemical composition, total antioxidant and anti-nutritional factors contents as well as studying their effects on obese anemic rats were investigated. Forty-eight rats were divided into two main groups. First main group (n=6 rats) was kept as normal group. Second one (n=42) were fed on high-fat diet with 10% tannic acid and iron-removed from the mineral mixture all the period to cause obese anemic rats and were divided into 7 subgroups (6 rats each). One of them was served as a positive control group (+ve), the other subgroups were supplemented with dried raw quinoa, germinated quinoa, soaked quinoa, fermented quinoa before soaking, fermented quinoa after soaking and quinoa cooked under pressure at the level of 20%, respectively. The different preparing methods significantly ($P<0.05$) increased the total antioxidant capacity as well as total flavonoids and phenols, in addition, decreasing the antinutritional factors. The highest weight reduction was recorded with germinated quinoa seeds. In addition, different types of quinoa preparation improved lipid profile, liver and kidney functions. Also, significant increase ($P<0.05$) in red blood cell counts (RBC), hematocrit (HCT), hemoglobin (Hb) and significant decrease ($P<0.05$) in white blood cell counts (WBC) concentrations, were observed as compared to the +ve group. The study recommends investigating consumption quinoa in different processing methods by obese anemic patients, especially the germinated and soaked quinoa.

Key words: Quinoa, obesity, anemia, germination, soaking, fermentation with or without soaking, cooking under pressure, rats.

Introduction

Anemia is the upper 20 risk reasons for the worldwide prevalence of disease responsibility (Hegde et al., 2006 and Mousa et al., 2016). WHO is working together with the Egyptian administration to report major challenges due to the prevalence of anemia among 40% of children between 2–5 years, which may increase to 51% in countryside children. The same trend was found between women of procreant age and in pregnancy (WHO, 2010; Abdel-Rasoul et al., 2017 and Simbrunner et al., 2020). Anemia has deleterious negative effects on maternal and fetal morbidity and

mortality, affects productive, mental education, work capability, resistance to infection, and pregnancy (**Helmy et al., 2018 and Means, 2020**).

Obesity, described as a body mass index (BMI) above 30 kg/m². Obesity prevalence will reach 50% by 2030 (**Ward et al., 2019**). The current prevalence of obesity is 42% and the severe adult obesity (BMI > 40) has doubled within the past 20 years (**Hales et al., 2020**). Obesity disproportionately burdens minorities, females, and lower socioeconomic status populations (**Ward et al., 2019**).

Quinoa seeds (*Chenopodium quinoa* Willd.) are a good source of 'functional food' that lowering a lot of diseases due to their vitamins, minerals, antioxidants and fatty acids content that protect cell membranes and confirmed best finding in neuronal functions of the brain (**Antonio et al., 2010**). Quinoa seed had nutritious grain due to providing high quantity and quality of proteins, essential fatty acids and dietary fibers (**Vega-Gálvez et al., 2010**). In addition, its minerals act as cofactors in antioxidant enzymes. These seeds also contain phytohormones, that is vital to human nutrition (**Antonio et al., 2010**). Quinoa is a good mineral source, it provides higher calcium, (Ca), magnesium (Mg) iron (Fe), and zinc (Zn) than other common cereals, and its content of Fe is particularly high. It contains also higher α -tocopherol and riboflavin (B2) than rice, wheat, or barley (**Jancurova et al., 2009**).

Quinoa seeds are gluten-free and a valuable source of high-quality protein, fibers, carbohydrates, vitamins, minerals, phytochemicals, and bioactive peptides. The antinutritional factors found in quinoa, can diminish the bioavailability due to forming insoluble complexes with minerals, which can be alleviated by germination (**Jarvis et al., 2017 and Vilcacundo and Ledesma, 2017**).

This study was conducted to evaluate the effect of different processing techniques on improving the nutritional quality of quinoa and their effect on body weight status and blood picture of obese anemic rats.

Materials and methods

The biological experiment and the chemical analysis were carried out at the Laboratories of the Regional Center for Food and Feed, Agricultural Research Center, Giza

Materials:

Quinoa: Twenty kilograms of quinoa seeds (*Chenopodium quinoa* Willd., var. *Real*) were taken from Crop Intensification Research Section Field Crops, Research Institute, Agriculture Research Center during August, 2019. **Chemicals:** Casein, Vitamins, Minerals, DL-methionine, starch and Cellulose were obtained from El-Gomhoria Company, Cairo, Egypt. Sucrose and oil were bought from local market. Tannic acid was purchased from local distributor of (Sigma Chemical Co) Cairo, Egypt. **Kits** for blood analysis was obtained from Alkan Company for Biodiagnostic Reagents, Dokki, Cairo, Egypt. **Rats:** forty-eight adult male Albino rats weighing from 120-130 gm were acquired from the animal house in Food Technology Research Institute, Cairo, Egypt.

Methods:

Quinoa Preparation:

Quinoa seeds were cleaned to remove dirt and other impurities. Stored in refrigerated multi-layer paper bags until prepared in one of the following four ways as follows, germination according to

(*Padmashree et al., 2019*), Soaking according to (*Park and Morita, 2005*), fermentation process: this method was performed on two samples of quinoa seeds, with or without soaking according to (*Valencia et al., 1999*) and cooking under pressure according to (*Júlia et al., 2016*).

Analytical Methods of Quinoa:

Dried Quinoa seeds were analyzed by standard methods for moisture, protein fat, ash and crude fiber according to **A.O.A.C, (2012)**. Total carbohydrate was calculated by difference. Gross energy was calculated by using the factors as described by **FAO/WHO/UNU, (1985)** according to the following equation:

Gross energy = 4 × (Protein % + Carb. %) + 9 × (Fat %).

Minerals and vitamins:

Magnesium (Mg), iron (Fe), calcium (Ca), and zinc (Zn) were determined according to the method of **A.O.A.C, (2012)** using Atomic Absorption Spectrophotometer, Perkin-Elmer Model 2380 manufacture, USA, also thiamin (B1) and riboflavin (B2) were determined according to **Hossain et al., (2010)**.

Total antioxidant capacity, total phenolic content (TPC) and total flavonoid content (TFC) of the samples were determined by the method of (**Prieto et al., 1999; Singleton et al., 1999 and Sarikurkcu et al., 2009**).

The saponin, phytic acid, alkaloids, tannins and oxalates content were determined following **Obadoni and Ochuko, (2001); Haugh and Lantzech, (1983); Harborne (1973); Makkar et al., (1993), and Abaza et al., (1968)**, respectively.

Experimental Animal Design:

Induction of obesity and anemia: Forty-eight adult meal albino rats survived in well-ventilated cages under hygienic condition according to healthy specifications and fed for a week on basic diet (**Reeves et al., (1993)**) for adaptation, then rats were distributed into two main groups. Group I major (**A**) (n=6) continued basal diet throughout the trial period (two months) and served as negative control group (-ve). The second main group (**B**) (n=42) was fed on basal diet with some modification for two months including (high fat diet) contains: Casein 14%, Cellulose 5%, Vitamin mixture 1%, Mineral mixture 3.5% without iron, Sucrose 10%, Beef tallow 19% + Soybean oil 1%, L-cystine 0.18%, Choline bitartrate 0.25%, Tannic acid 10 g/kg diet and the rest is starch to induce obesity according to (**Liu et al., 2004**) and to cause anemia in rats according to **Afsana et al., (2004)**. In normal rats the hemoglobin (Hb) concentration was registered to be (13.53±0.58 g/dl) (**Wolford, et al., 1986**). That group (B) was divided into (7) subgroups, six rats each as follow:

- Subgroup (B1) was served as a positive control group (+ve) till the end of the experimental period. Other 6 subgroups from (B2:B7) were divided as follows: -
- Subgroup (B2) was fed a high-fat diet without iron in minerals and adding 10% tannic acid supplemented with 20% dried Raw quinoa.
- Subgroup (B3) fed a high-fat diet without iron in minerals and adding 10% tannic acid supplemented with 20 % dried germinated quinoa seeds.
- Subgroup (B4) fed a high-fat diet without iron in minerals and adding 10% tannic acid supplemented with 20 % of dried soaked quinoa seeds.

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- Subgroup (B5) fed a high-fat diet without iron in minerals and adding 10% tannic acid supplemented with 20 % of dried fermented quinoa seeds before soaking.
- Subgroup (B6) fed a high-fat diet without iron in minerals and adding 10% tannic acid supplemented with 20 % of dried fermented quinoa seeds after soaking.
- Subgroup (B7) fed a high-fat diet without iron in minerals and adding 10% tannic acid supplemented with 20 % of dried quinoa seed cooked under pressure.

Finally (8 weeks) the rats were fasted overnight before collected two blood samples, one of which was obtained from the medial throat of mice eyes via micro-glass tubes and collected in a tube containing EDTA as an anticoagulant and used for blood picture parameters determination. Another blood sample was collected in a centrifuge tube without any anticoagulant and centrifuged for 20 min at 3000 rpm to obtain serum which was stored at -20°C until subsequent biochemical analysis.

Biological Evaluations:

The amounts of food consumed and/or wasted, were recorded every day while total feed intake (FI) was calculated. In addition, body weight (BW) of rat's was recorded weekly. Body weight gain percentage (BWG%) and feed efficiency ratio (FER) were calculated according to **Champan, et al., (1959)** using the next equation:

$$\text{BWG}\% = \frac{\text{Final body weight (FBW)} - \text{Initial body weight (IBW)}}{\text{Initial body weight}} \times 100$$
$$\text{FER} = \frac{\text{Body weight Gain (BWG) (g)/day}}{\text{Feed intake (FI) (g)/day}}$$

Biochemical Evaluations:

Red blood cell counts, HCT, Hb and counts of WBC were determined according to (**Dacie and Lewis, 1991 and Alexander and Griffiths, 1993**) respectively. Serum total cholesterol (TC), high density lipoprotein cholesterol (HDL-c) and triglyceride (TG) were determined by (**Fossati and Principe 1982; Albers et al., 1983 and Jacobs and Vander, 1960**), respectively. Calculations of low-density lipoprotein cholesterol (LDL-c) and very low-density lipoprotein cholesterol (VLDL-c) by the equation of **Fruchart (1982)**. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured according to **Reitman and Frankel (1957)**. Serum albumin was measured according to (**Weissman et al., 1950**).

Statistical analysis:

Results was communicated as mean± standard Error (SE). Data were analyzed statistically by SPSS program, one-way ANOVA followed by post hoc multiple were used to make a comparison among different groups **Snedecor and Cochran, (1989)**.

Results and Discussion

**Table (1):
The gross chemical composition of different processing for quinoa seeds:**

Parameter Processing	Protein	Ash	Moisture	Fat	Fiber	Carb.	Gross Energy
	%				kcal/100 g		
Raw seed	15.5 ±0.12 ^d	4.00 ±0.58 ^a	5.10 ±0.06 ^{ab}	5.26 ±0.0 ^a	3.89 ±0.06 ^{ab}	66.25 ±0.25 ^{ab}	374.34 ±0.12 ^c
Germinated seeds	18.9 ±1.15 ^{ab}	2.50 ±0.17 ^{bc}	4.60 ±1.27 ^b	4.74 ±0.06 ^{ab}	5.23 ±0.06 ^a	64.03 ±2.25 ^b	374.38 ±0.06 ^c
Soaked seeds	17.1 ±0.06 ^{cd}	1.80 ±0.12 ^d	6.00 ±1.73 ^{ab}	5.07 ±0.04 ^a	5.70 ±0.06 ^a	64.33 ±2.06 ^{ab}	371.35 ±0.12 ^d
Fermented seeds without soaking	19.4 ±0.6 ^a	2.20 ±0.12 ^{bc}	6.30 ±0.58 ^{ab}	5.17 ±0.87 ^a	3.90 ±0.64 ^{ab}	63.03 ±0.23 ^b	376.25 ±0.29 ^b
Fermented seeds after soaking	15.6 ±0.35 ^d	2.50 ±0.03 ^{cd}	7.90 ±0.64 ^a	3.5 ±0.17 ^b	3.23 ±0.12 ^b	67.19 ±0.37 ^{ab}	362.66 ±1.15 ^e
Seed cooked under pressure	17.7 ±0.06 ^{bc}	2.80 ±0.03 ^b	4.07 ±0.06 ^b	5.85 ±0.61 ^a	4.71 ±0.58 ^a	68.24 ±0.09 ^a	396.41 ±0.06 ^a

Data are expressed as mean ± SE.

Means with different superscript letters in the column are significantly differences at ($P \leq 0.05$).

There were significant differences in nutritive composition of quinoa due to different processing. As shown in table (1) protein content (15.5%) and total carbohydrates (66.25 %) were relatively high. The recorded values for fat, ash and fiber were 5.26, 4.00 and 3.89 %, respectively in raw quinoa seeds. These results agreed with those by (*Vilcacundo and Hernandez-Ledesma, 2017 and Wahba et al., 2019*). Protein and carbohydrate content of quinoa were relatively high compared with other breakfast cereal like rice and wheat. Moreover, quinoa seeds of high protein quality since it provides with many essential amino acids which meet the requirements for adults as stated by (*WHO, 2010*).

Germination of quinoa showed a statistical increase ($p \leq 0.05$) on the protein content from 15.5 to 18.9%. The increase level in fiber content by 34.4% as compared with quinoa seeds were recorded in the same table.

This improvement may be due to the loss of dry matter especially carbohydrate through respiration during germination (*Uppal and Bains, 2012*) or the reawakening of protein synthesis upon imbibition (*Nonogaki et al., 2010*) which may lead to the increased protein content. Moisture and lipid content had significant decrease from 5.1% to 4.6% and 5.26 to 4.74 %, respectively. These results coincided with those of *Jan et al., (2017)*, who noted a significant decrease in fat content may be due to its use as a source of energy during germination. Significant decrease in ash content from 4.0 to 2.5 %. *Chinma et al., (2009)* pointed out that the lower ash content through germination could be due to leaching out of some water- sensitive soluble minerals during soaking.

At soaking process, there has been a significant increase in protein and fiber content by 17.1 and 5.7% respectively compared to the (- ve) group. Other side, there was a significant decrease in carbohydrate, ash and fat by 64.33%, 1.8% and 5.07%, respectively.

Results in a table (1) also showed that the fermentation process without seed soaking was better than the fermentation process with seed soaking in its effect on the chemical composition of quinoa seeds. Fermented quinoa seeds without soaking had higher amounts of protein (19.4%) as evidenced by value significantly increased to 25.1%. Also represented a good source of fiber (3.90%) with only 63.03% and 5.17% of daily values of carbs and fats. These results were consistent with **(Songlin et al., 2018)** who stated that the fermentation with *Lactobacillus casei* on a lot of parameters of quinoa seeds like protein, free amino acids, carbohydrate, ash were statistically ($p < 0.05$) higher than quinoa seeds. The significant decrease in carbohydrate content after germination from 66.25 to 64.03 % can be attributed to its utilization as a source of energy for the growth of embryos during the germination process **(Ferreira et al., 2009)**. The same findings were mentioned by **(Pilco-Quesada et al., 2020)**. While some researchers reported that the germination of quinoa seeds significantly decreased protein content by 11% from 15.13 to 13.50 g/100 g **(Darwish et al., 2020)**.

Pressure-cooked quinoa seeds showed significantly more of all nutritional composition except for ash and moisture (4.0 to 2.8 and 5.1 to 4.07, respectively than raw quinoa seeds. These results are agreement with **(FAO, 2011)** that protein content quinoa ranged between 13.81 and 21.9% depending on the type or treatments made on it, and our results (17.7%) are identical to this study, moreover, quinoa contains a high percentage of fiber (Table 1), which makes it a valuable food to get rid of toxins and waste that may harm the body.

Bhathal et al., (2017) proved the flour given from raw quinoa had higher amounts of crude protein (14.02%), crude fat (5.13%) and total ash (3.83%). Also, **Darwish et al., (2020)** revealed that quinoa seeds provided energy of 371.18 Kcal/100g, fiber and protein (43.08% and 30.62%), respectively, with 20.47% and 10.98% of carbs and fats. Numerous studies have confirmed the high quality of protein in quinoa that gives all kinds of essential amino acids **(Comino et al., 2013 and Dakhili et al., 2019)**.

Filho et al., (2017) reported high digestibleness of quinoa protein. Raw quinoa provides 91.6% proteins, and the quantity can be further raised to 95.3% by heat therapy **(Ruales et al., 2002)**. Due to this high digestibility, the biological value of quinoa (73%) is like beef (74%) **(Gordillo-Bastidas et al., 2016)**.

In addition, quinoa is a gluten free grain that patients with celiac disease can safely eat **(Comino et al., 2013)**. The lipid content of quinoa has been reported from 5.3% to 14.5%, characterized by a high degree of unsaturation ranging from 70% to 89.4% **(Gordillo-Bastidas et al., 2016)**. It contains 8– 13% dietary fiber **(Tanwar et al., 2019)**. Quinoa fiber composition varies with different growth conditions and genotypes, with insoluble fiber from 10% to 14%, and soluble fiber ranging from 1.3% to 6.1% **(Zhu, 2020)**.

Table (2):
The Effect of different processing on quinoa seeds:

Processing \ Parameter	Total antioxidant capacity (mg/100g)	Total flavonoids (mg/100g)	Total phenols compound (mg/100g)
Raw seed	410.64 ±3.24 ^c	123.65±0.34 ^b	201.7±0.09 ^e
Germinated seeds	1013.9±0.86 ^a	161.7±0.57 ^a	347.8±0.24 ^a
Soaked seeds	249.43±0.57 ^e	123.42±0.63 ^b	162.3±0.17 ^f
Fermented seeds without soaking	327.7±0.60 ^d	90.44±0.02 ^c	313.5±0.57 ^c
Fermented seeds after soaking	253.12±0.06 ^e	51.94±0.02 ^e	225.6±0.88 ^d
Seed cooked under pressure	737.00±0.57 ^b	71.79±0.05 ^d	318.3±0.88 ^b

Data are expressed as mean ± SE. Means with different superscript letters in the column are significantly differences at ($P \leq 0.05$).

Total antioxidant expressed as ascorbic acid equivalent, total flavonoids expressed as Quercetin equivalent and total phenols expressed as gallic acid equivalent.

Table (2) recorded the total antioxidant capacity, total flavonoids and total phenols of quinoa seeds affected by different processes. The germination and cooking under pressure significant increased ($P < 0.05$) in total antioxidant capacity compared to the raw quinoa seed (from 410.64 "raw" to 1013.9 "for germination" and 737 mg/100g "for the cooked under pressure"). The same results have been achieved with phenolic compounds that's recorded (from 201.7 to 347.8 and 318.3mg/100g).

Results concurred with those of (*Júlia et al., 2016*) who mentioned that the antioxidant capacity was increased in the cooked grains, especially when cooked with pressure. Furthermore, the increases examined of total phenolic compound through germination could be characterized by the action of endogenous esterase that is synthesized during germination which can lead to the release of cell wall bound phenolic compounds. Also, the biochemical reactions in grain germinating could lead to the synthesis of new phenolic compounds (*Singh et al., 2015*).

Non-significant change was shown in total flavonoids of soaked seeds compared to raw seeds, while the other processing showed significant decrease.

The predominant polyphenols in quinoa are flavanols-type flavonoids, including quercetin, kaempferol, and their derivation (*Balakrishnan and Schneider, 2020*). Other studies reported that germination significantly increased the total phenolic content in quinoa (*Al-Qabba et al., 2020; Darwish et al., 2020 and Bhinder et al., 2021*). The total phenolic content of red and yellow quinoa increased by further than 200% after 6 days of germination, and slightly decreased (*Al-Qabba et al., 2020*).

Functional constituents that contribute to the antioxidant capacity of quinoa might be the phenolic composites including flavonoids (*Ahmed et al., 2020*). The germination process could offer a

good strategy to improve the antioxidant activity properties of the seeds (*Smolikova et al., 2011; Darwish et al., 2020 and Ruijuan et al., 2021*).

**Table (3):
Effect of different processing on anti-nutritional factors in quinoa seeds:**

Processing	Parameter	Saponin	Alkaloids	Oxalate	Phytic acid	Tannins
						%
Raw seed		2.84±0.01 ^a	0.735±0.0 ^a	0.209±0.0 ^a	1.3555±0.0 ^a	0.49±0.02 ^a
Germinated seeds		1.272±0.0 ^f	0.185±0.0 ^f	0.130±0.0 ^e	1.2648±0.0 ^f	0.18±0.01 ^d
Soaked seeds		1.766±0.0 ^e	0.235±0.0 ^e	0.154±0.0 ^d	1.2675±0.0 ^e	0.25±0.02 ^c
Fermented seeds without soaking		2.04±0.01 ^d	0.56±0.01 ^b	0.176±0.0 ^c	1.3117±0.0 ^d	0.35±0.01 ^b
Fermented seeds after soaking		2.596±0.0 ^c	0.41±0.02 ^c	0.198±0.0 ^{ab}	1.3251±0.0 ^c	0.33±0.02 ^b
Seed cooked under pressure		2.676±0.0 ^b	0.365±0.0 ^d	0.187±0.0 ^{bc}	1.3358±0.0 ^b	0.34±0.01 ^b

Data are expressed as mean ± SE.

Means with different superscript letters in the column are significantly different at (P ≤ 0.05).

Quinoa seeds contain many bioactive components, including saponins, terpenes, and flavonoids which seems to be in accordance with our findings (*Navruz-Varli and Sanlier, 2016; Vilcacundo and Hernandez-Ledesma, 2017 and Wahba et al., 2019*). Among the phytochemical compounds in quinoa seeds, it was said to contain phytosterols and phytoecysteroids (*Villacrés et al., 2013 and Graf et al., 2015*).

The study showed that all different processing decreased significantly the saponins, alkaloids, oxalate, phytic acid and tannins compared to the raw seed. Moreover, the highest reduction in the antinutritional factors is observed at the germination process followed by soaking method.

This supported the work of *Darwish et al., (2020)* who found that germination method decreased the antinutritional factors, saponins, tannins and phytic acid. Saponins is lowered due to their leaching from quinoa seeds during washing and soaking (*Bhathal et al., 2015*). Phytic acid content significantly lowered due to its break down as source of phosphorus for utilization during germination (*Padmashree et al., 2019*). The same trend was observed in tannins content due to enzymatic changes during the germination period (*Megat Rusydi and Azrina, 2012*).

Saponins and phytic acid, with some other antinutrients such as tannin, trypsin inhibitor, and oxalate present in trace quantities are found in quinoa seeds (*Bhargava and Srivastava, 2013*). Some saponins are considered antinutritional because they caused insoluble complexes with some minerals (*Caballero et al., 2003*). Phytic acid is an antinutrient commonly found in plant-source food, which exerts strong chelating activity on minerals or other positively charged molecules. However, germination significantly reduce these antinutritional components and increase the mineral content of

quinoa (*Al-Qabba et al., 2020; Darwish et al., 2020 and Bhinder et al., 2021*). In addition, saponins are usually removed from the seed before consumption as a result of their bitter taste (*Al-Qabba et al., 2020*). Recently, *Landi et al., (2021)* reported a protein toxin found in quinoa, called, quinoan, which can prevent protein synthesis and was found to provoke cell morphological alternations. In addition, to consume cooked quinoa instead of raw quinoa, because protein toxins are easily denatured at high temperature. Soaking for 12–18 h reduce the levels of proteolytic enzyme inhibitors as well as phytic acid, which are partly or wholly solubilized in soaked water (*Kajihansa et al., 2014*). Moreover, soaking and germination are good methods to lowering the anti-nutritional component and improve the nutritional, bioactive, and antioxidant potential of quinoa (*Thakur et al., 2021*).

Table (4):
Effect of different processing for quinoa seeds on its content of Vit. B1 (Thiamine) and Vit. B2 (Riboflavin).

Processing	Vitamins	Vit B1 mg/100g	Vit B2 mg/100g
Raw seed		0.530±0.01 ^a	0.160±0.01 ^a
Germinated seeds		0.510±0.01 ^b	0.048±0.01 ^d
Soaked seeds		0.510±0.01 ^b	0.082±0.00 ^c
Fermented seeds without soaking		0.300±0.00 ^c	0.140±0.01 ^{ab}
Fermented seeds after soaking		0.170±0.01 ^d	0.077±0.00 ^c
Seed cooked under pressure		0.140±0.01 ^e	0.130±0.01 ^b

Data are expressed as mean ± SE.

Means with different superscript letters in the column are significantly differences at (P ≤ 0.05).

Data in table (4) indicated the content of vitamins (B1 and B2) in raw quinoa seeds and quinoa seeds affected by germination, soaking, fermentation and cooking under pressure. *Gordillo-Bastidas et al., (2016) and Schoenlechner, (2017)* reported that, quinoa is a good source of some vitamins including thiamine, folic acid, vitamin B6 and vitamin B5. Vitamin B1 content significantly decreased under all processed groups as well as vitamin B2 content while the treatments by fermentation without soaking caused non-significant changes in B2 content as compared with raw seeds.

Table (5):
Effect of different processing for quinoa seeds on its contents of minerals:

Processing	Minerals	Zn	Fe	Mg	Ca
		mg/100g			
Raw seed		1.68±0.01 ^e	13.06±0.05 ^b	130±1.0 ^b	70±1.73 ^c
Germinated seeds		2.18±0.01 ^c	17.61±0.35 ^a	140±1.15 ^a	90±2.64 ^a
Soaked seeds		1.15±0.01 ^f	3.07±0.01 ^f	60±0.57 ^f	40±2.64 ^d
Fermented seeds without soaking		3.12±0.01 ^a	9.02±0.01 ^c	120±1.15 ^c	80±1.73 ^b
Fermented seeds after soaking		2.31±0.01 ^b	8.024±0.01 ^d	100±0.57 ^d	40±1.52 ^d
Seed cooked under pressure		1.84±0.01 ^d	6.39±0.003 ^e	80±0.57 ^e	40±1.15 ^d

Data are expressed as mean ± SE.

Means with different superscript letters in the column are significantly differences at (P ≤ 0.05).

Minerals contents are illustrated in Table 5 and showed that germination process was the best process which increased the levels of zinc, iron, magnesium and calcium contents in quinoa seeds by 29.6, 34.8, 7.7 and 28.6%, respectively than other levels of raw seeds. This means that; germination of quinoa seeds enhanced the minerals content and improved the nutritional values of quinoa.

Quinoa was rich in minerals as bioavailable forms (*Gordillo-Bastidas et al., 2016*). Germination enhances the iron, calcium, and zinc content of quinoa by 39.43%, 49.04%, and 20.25%, respectively (*Darwish et al., 2020*). *Welch and Graham, (2004)* found that high phytic acid levels greatly prevent iron bioavailability and that germination decreased phytic acid, so it increased bioavailability of minerals such as iron and zinc (*Benincasa et al., 2019*).

Table 6:
Effect of different types of quinoa preparation on body weight of obese anemic rats.

Parameters Groups	IBW (g)	FBW (g)	BWG %	FI (g/day/rat)	FER
Control -ve	153.6±0.88 ^b	171.8±4.4 ^b	11.87±3.40 ^b	15.00	0.020±0.05 ^b
Control +ve	188.5±5.77 ^a	238.9±0.74 ^a	26.94±3.47 ^a	21.30	0.039±0.04 ^a
Raw seed	190.8±3.34 ^a	175.0±2.51 ^b	-8.20±2.79 ^c	17.00	-0.015±0.05 ^c
Germinated seeds	192.1±3.05 ^a	168.4±0.80 ^b	-12.31±1.29 ^c	15.60	-0.025±0.03 ^c
Soaked seeds	190.0±4.04 ^a	174.2±3.09 ^b	-8.28±1.30 ^c	17.00	-0.015±0.02 ^c
Fermented seeds before soaking	192.1±4.20 ^a	174.6±2.90 ^b	-9.07±1.06 ^c	17.20	-0.017±0.02 ^c
Fermented seeds after soaking	192.9±2.70 ^a	170.7±2.25 ^b	-11.44±1.81 ^c	16.40	-0.022±0.03 ^c
Seed cooked under pressure	188.7±3.34 ^a	170.8±1.36 ^b	-9.40±1.96 ^c	16.50	-0.018±0.04 ^c

Data are expressed as mean ± SE.

Means with different superscript letters in the column are significantly differences at ($P \leq 0.05$).

The IBW was recorded after the induction of obesity, so that it was significantly higher in all groups as compared to the (-ve) group. The statistical analysis showed that the mean values of body weight, BWG %, FI and FER of (+ve) group were significantly ($P < 0.05$) increased, compared to the (-ve) group. Obese anemic rats treated with different types of quinoa preparation had significant ($P < 0.05$) decrease in final body weight, BWG%, FER, compared to (+ve) group (obese anemic rats). Moreover, there were no significant differences in levels of IBW, BWG%, FI and FER among different groups treated with different types of quinoa preparation. The highest decrease in the final body weight, BWG% and FER was recorded by treatment with germinated quinoa seeds.

Few studies demonstrated the anti-obesity potential of quinoa polysaccharide (*Cao et al., 2020; Teng et al., 2020 and Ng and Wang, 2021*). High-fat-diet induced obese rats supplemented with quinoa polysaccharide showed a lower level of fat accumulation in adipocyte compared with the +ve control group (*Cao et al., 2020*). A purified polysaccharide from quinoa inhibited the differentiation of adipocytes by suppressing the expression of several genes related to adipogenesis (*Teng et al.,*

2020). In addition, the inhibitory effect of phenolic compounds on digestive enzymes helps decrease the absorption of energy (Noratto et al., 2019). Fotschki et al., (2020) proved that the body weight of rats fed with diets containing quinoa protein-rich flour was lower than that of the (+ ve) group.

Wahba et al., (2019) showed that quinoa seed powder at different levels (10, 20, 30 and 40%) showed a significant improvement in each of BWG, FI and FER compared to the (+ve) group. Vega-Gálvez, (2010) reported that quinoa is a very nutritious source because it contains a good balance of carbohydrates, lipid, fiber, amino acids, minerals and vitamins. Quinoa seeds work as anti-obesity activities and could be used as a nutritional complement for treating and preventing disorders of obesity (Hejazi, 2016). Studies have reported a significant decrease in BWG in rats fed on high cholesterol-diet supplemented with quinoa (Halaby et al., 2017 and Alghamdi, 2018), high-sugar-diet-induced rats (Lopes, et al., 2018 and Mohamed et al., 2019), healthy rats (Fotschki et al., 2020), and healthy humans (Pourshahidi et al., 2020).

The weight loss effect of quinoa appeared more in population on a high-fat diet. The potential mechanisms of quinoa's anti-obesity ability are inhibition of adipocyte differentiation through gene regulation, increasing energy expenditure, and lowering fat absorption. furthermore, quinoa can be used as a good nutrient source for body recovery (Simnadis et al., 2015 and Ng and Wang, 2021).

Table 7:
Effect of different processing for quinoa seeds on lipid profile of obese anemic rats.

Parameters	HDL-C	TG	TC	VLDL-C	LDL-C
	(mg/dl)				
Control -ve	60.93±1.65 ^a	73.43±1.82 ^d	87.83±1.69 ^d	14.68±0.36 ^d	12.21±1.66 ^d
Control +ve	27.87±2.22 ^c	110.93±3.15 ^a	128.76±1.13 ^a	22.18±0.63 ^a	78.71±2.12 ^a
Raw seed	44.34±1.82 ^b	87.53±4.29 ^c	99.80±3.06 ^c	17.50±0.85 ^c	37.71±4.77 ^{bc}
Germinated seeds	50.69±1.19 ^b	88.56±1.54 ^c	98.50±5.39 ^{cd}	17.71±0.30 ^c	30.09±4.45 ^c
Soaked seeds	41.47±1.18 ^b	98.96±4.16 ^b	114.40±2.69 ^b	19.79±0.83 ^b	53.13±2.60 ^b
Fermented seeds before soaking	43.49±1.38 ^b	84.20±3.81 ^c	100.16±3.14 ^c	16.84±0.76 ^c	39.83±4.09 ^{bc}
Fermented seeds after soaking	49.83±4.90 ^b	84.20±3.18 ^c	100.56±5.48 ^c	16.84±0.63 ^c	33.89±9.43 ^c
Seed cooked under pressure	42.39±5.52 ^b	94.90±3.12 ^{bc}	103.20±3.86 ^c	18.98±0.62 ^{bc}	41.82±4.89 ^{bc}

Data are expressed as mean ± SE.

Means with different superscript letters in the column are significantly differences at (P ≤ 0.05).

Results in Table (7) indicated that obese anemic rats treated with different types of quinoa preparation had significant decrease (P<0.05) in serum levels of TC, TG, VLDL-c, LDL-c and had a significant increase in serum HDL-c, as compared to the (+ ve) group.

There are no significant differences for HDL-c among all treated groups. Moreover, the same trend was observed for serum TG, VLDL-c, TC, and LDL-c among the groups treated with either

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fermented seeds, fermented seeds before or after soaking or cooking under pressure. The best improvement of lipid profile is recorded for obese anemic rats treated with germinated quinoa seeds and fermented quinoa seeds after soaking followed by fermented quinoa seeds before soaking

Graf et al., (2015) mentioned that the marked hypocholesterolemic effect of quinoa may be attributed to its content of saponins. Moreover, the fiber content of quinoa may contribute to its hypolipemic effect. **Navarro-Perez et al., (2017)** found that chronic consumption of quinoa might help reduce the risk of cardiovascular disease. **Halaby et al., (2017) and Alghamdi, (2018)** illustrated the positive effects of quinoa on cardiovascular health. The consumption of quinoa can improve lipid profile, including a significant decrease in TG, LDL-C level, and caused a significant increase in HDL-C level (**Lopes, et al., 2018; Ali, 2019; Mohamed et al., 2019; Wahba et al., 2019; Al-Qabba et al., 2020 and Abdel-Wahhab et al., 2021**).

Although the results of various studies could be different, the effects of quinoa consumption on the lipid profile showed a similar trend. Recent evidence supports that saponins have hypocholesterolemic activity (**Marrelli et al., 2016 and Singh et al., 2017**). **Simnadis et al., (2015)** investigated physiological effect of consuming quinoa seeds to decrease weight gain and improve lipids profile. **Ali, (2019)** reported that all supplemented groups with quinoa seeds 5 and 10%, had significant increase in HDL-C, while serum, TC and LDL-C were decreased significantly.

Table 8:
Effect of different processing for quinoa seeds on blood picture in obese anemic rats.

Parameters	RBC (million/c.mm)	HCT %	WBC (Th.c.mm)	Hb %
Control -ve	7.90±0.20 ^a	69.6±0.88 ^a	4.03±0.20 ^f	13.76±0.46 ^a
Control +ve	4.70±0.35 ^d	40.7±0.88 ^d	7.20±0.23 ^a	7.83±0.42 ^d
Raw seed	6.30±0.35 ^c	51.2±2.26 ^c	5.90±0.05 ^{bcd}	10.06±0.58 ^c
Germinated seeds	7.53±0.08 ^a	62.9±1.98 ^b	4.63±0.34 ^{ef}	12.10±0.58 ^b
Soaked seeds	6.10±0.50 ^c	50.6±1.96 ^c	6.00±0.11 ^{bc}	9.93±0.48 ^c
Fermented seeds before soaking	6.46±0.29 ^{bc}	54.0±3.21 ^c	5.40±0.23 ^{cd}	10.26±0.37 ^c
Fermented seeds after soaking	7.46±0.14 ^{ab}	61.3±1.39 ^b	5.20±0.28 ^{de}	11.76±0.27 ^b
Seed cooked under pressure	6.03±0.48 ^c	49.5±1.51 ^c	6.13±0.17 ^b	9.77±0.56 ^c

Data are expressed as mean ± SE.

Means with different superscript letters in the column are significantly differences at ($P \leq 0.05$).

Effect of different types of quinoa preparation on RBC, HCT, WBC and Hb levels of obese anemic rats are recorded in Table (8). Results revealed that (+ve) group had significant decrease ($P < 0.05$) in RBC, HCT, Hb and caused significant increase in WBC counts of obese anemic rats compared to the -ve control group.

However, obese anemic rats treated with different types of quinoa preparation had significant increase ($P < 0.05$) in RBC, HCT, Hb but caused significant decrease ($P < 0.05$) in WBC counts, compared to the +ve group. Moreover, obese anemic rats treated with germinated quinoa seeds or fermented seeds after soaking had the highest increase ($P < 0.05$) in RBC parameters concentrations and the lowest decrease in level of WBC compared to other treatments. Moreover, there were non-significant changes in levels of RBC, HCT, WBC and Hb among all groups treated with quinoa preparation (soaked, fermented before or after soaking).

Antianemic impact of quinoa seeds and sprouts was documented (*Ibrahim, 2015 and Manikandaselvi et al., 2015*). *Darwish et al., (2020)* indicated that 10% quinoa sprouts restore the values of the blood indices (RBC, HCT and Hb) to the normal levels. Moreover, the levels of antioxidants, vitamins, minerals content, and bioavailability of nutritional compounds are increased due to lowered antinutritional factors content in the germinated sprouts.

Table 9:
Effect of different processing for quinoa seeds on liver functions of obese anemic rats.

Parameters Groups	ALT	AST	ALP
	/L μ		
Control -ve	27.66±1.05 ^d	70.0±0.57 ^d	107.8±5.49 ^d
Control +ve	66.13±2.31 ^a	111.4±1.92 ^a	217.6±1.87 ^a
Raw seed	47.00±6.11 ^b	86.7±1.66 ^{bc}	147.5±3.04 ^b
Germinated seeds	36.76±1.76 ^c	91.3±5.54 ^{bc}	127.5±1.81 ^c
Soaked seeds	39.56±0.29 ^{bc}	91.6±2.72 ^c	118.3±1.85 ^c
Fermented seeds before soaking	36.13±1.13 ^c	84.0±3.05 ^c	121.0±1.00 ^c
Fermented seeds after soaking	44.77±2.33 ^{bc}	92.0±2.00 ^{bc}	125.3±4.37 ^c
Seed cooked under pressure	43.83±2.04 ^{bc}	96.7±5.92 ^b	139.0±1.15 ^b

Data are expressed as mean \pm SE.

Means with different superscript letters in the column are significantly differences at ($P \leq 0.05$).

Table (9) showed the effect of different types of quinoa preparation on liver functions of anemic obese rats. Supplementation with different types of quinoa preparation caused significant decrease ($P < 0.05$) in serum levels of AST, ALT and ALP compared to the (+ve) group. Moreover, there were no significant differences in serum levels of AST and ALT among all groups' different types of quinoa preparation. The best improvement of liver functions was recorded for obese anemic rats treated with fermented quinoa seeds before soaking for serum ALT and AST and soaked quinoa seeds for ALP.

Repo-Carrasco-Valencia, (2010) showed that treated groups with quinoa seeds revealed the greatest decline in serum liver enzyme compared with the +ve group. The results agreed with the study of *Ali, (2019)*, who assessed the antioxidant role of quinoa seeds. Phenolic compounds and fat-soluble vitamins, in quinoa may help reduce oxidative stress, by protecting the liver from oxidative damage (*Ali, 2019 and Ahmed et al., 2020*). In addition, quinoa polysaccharide might also contribute to liver health, due to reversing the hepatic steatosis in high-fat-diet-induced rats (*Cao et al., 2020*). Many animal studies have informed the hepatoprotective role of quinoa diet, notably under biological stress. Quinoa diet improved liver function in rats fed high-cholesterol diet (*Halaby et al., 2017 and Alghamdi, 2018*), *CCl4-induced rats (Saxena et al., 2017 and Al-Qabba et al., 2020)*, *high-fructose-diet induced rats (Mohamed et al., 2019)*, cyclophosphamide-poisoned rats (*Wahba et al., 2019*), and healthy rats (*Fotschki et al., 2020*), mainly supported by the significant reduction in serum ALT and AST levels. The lower of these indicators caused by quinoa consumption is considered useful to liver function (*Ng and Wang, 2021*).

Using different methods to preparing quinoa before adding it in diets, may be benefit in fighting obesity and human anemia and its complications. Also, we can eat quinoa by many ways such as a rice replacement, a hot breakfast cereal, boiled in water for making infant cereal food. As well, the

seeds can be popped like popcorn, grind to use as flour, or sprouted. Finally, we recommend the use of dried germinated quinoa seeds as a food ingredient in the formulation of valuable functional foods.

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تأثير العمليات التحضيرية المختلفة على تحسين القيمة الغذائية للكينوا ودراسة تأثيرها على الفران المصابة بالسمنة والانيميا

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الملخص العربي

نبات الكينوا هو من الحبوب ذات قيمة غذائية كبيرة وقد اكتسبت أهمية في معظم البلدان. تمر بذور الكينوا بالعديد من العمليات التي قد تؤثر على قيمتها الغذائية قبل الاستهلاك. لذلك قمنا بدراسة تأثير عمليات (الإنبات ، النقع ، التخمير مع أو بدون النقع والطهي تحت الضغط) على التركيب الكيميائي و مضادات الأكسدة و مضادات التغذية بالإضافة إلى دراسة تأثيرها على الفران المصابة بالسمنة والانيميا . تم تقسيم عدد 48 من ذكور الفران البالغة إلى مجموعتين رئيسيتين. تم الاحتفاظ بالمجموعة الرئيسية الأولى (n=6) كمجموعة ضابطة سالبة. تم تغذية المجموعة الرئيسية الثانية (n=42) على نظام غذائي عالي الدهون بالإضافة الي 10% حمض التانيك كما تم إزالة عنصر الحديد من خليط الاملاح المعدنية طوال فترة التجربة لإحداث السمنة والانيميا لدى الفران ، ثم قُسمت إلى سبع مجموعات فرعية (6 فران لكل منهم). تم إختيار إحداهم كمجموعة ضابطة موجبة (+ve) ، ثم قُسمت المجموعات الفرعية الأخرى وُعذبت بالكينوا (الخام المجففة، المنبته، المنقوعة ، المخمرة قبل النقع أو المخمرة بعد النقع والكينوا المطبوخة تحت ضغط) بنسبة 20 %، على التوالي.

أوضحت النتائج المتحصل عليها أن طرق التحضير المختلفة أدت الي زيادة معنوية ($P<0.05$) في نسبة مضادات الأكسدة الكلية وكذلك مركبات الفلافونويد والفينولات الكلية ، بالإضافة إلى تقليل العوامل المضادة للتغذية. تم تسجيل أعلى إنخفاض في الوزن للمجموعة الفرعية التي تغذت علي بذور الكينوا المنبته. بالإضافة إلى ذلك، فإن استخدام بعض من الطرق التحضيرية المختلفة للكينوا أدى الي تحسن ملحوظ في مستوى الدهون ووظائف الكبد والكلية . كما لوحظ زيادة معنوية ($P<0.05$) في كرات الدم الحمراء ونسبة الهيماتوكريت والهيموجلوبين وإنخفاض معنوي ($P<0.05$) في تركيزات كرات الدم البيضاء مقارنة مع مجموعة +ve. توصي الدراسة بتناول الكينوا بعد إجراء العمليات التحضيرية المختلفة عليها وذلك للحد من الإصابة بالسمنة والانيميا. كما يوصى دائماً بنقع الكينوا أو انباتها بدلاً من استخدامها في صورتها الخام لأنها تحسن من قيمتها الغذائية واستخدامها في عمل المنتجات الغذائية .