Evaluation of Phytochemicals and Antioxidant Activity of Gamma Irradiated Quinoa (*Chenopodium quinoa*)

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Received: December 17, 2019 – Accepted: February 7, 2020 – Distributed: August 31, 2021

With 3 figures

Abstract

Phytochemical and antioxidant activity of quinoa flour was evaluated after subjected to gamma irradiation processes at dose 3 and 6 kGy. Both non-irradiated and irradiated quinoa samples were subjected to successive extractions in ethanol solvent. The antioxidant activity after gamma irradiation treatment was investigated via Ferric reducing antioxidant power (FRAP) and radical-scavenging activity (RSA) using 2,2-diphenyl-1-picrylhydrazyl (DPPH). Total phenolic and flavonoid content were analyzed using Folin–Ciocalteu micro-method, aluminium chloride (AlCl₃) method and High Performance Liquid Chromatography (HPLC). As well as, effect of irradiation treatment on saponin was also evaluated. Irradiation treatment showed slight differences in the saponin content after exposure to 3 and 6 kGy. Irradiation process enhanced both total phenolic content (TPC) and Total flavonoid content (TFC), TPC were 34.52 and 30.92 mg Gallic Acid Equivalent (GAE)/100g compared to 26.25 mg GAE/100g in non-irradiated quinoa. TFC were 67.44 and 62.89 mg Quercetin Equivalents (QE)/100g compared to 53.15 mg QE/100g. Irradiation dose 3 kGy significantly (p> 0.05) decreased the IC₅₀ as DPPH-RSA and increased the FRAP.

Keywords: *Chenopodium quinoa*, Gamma Irradiation, polyphenols, antioxidant activity, saponin.

1. Introduction

So many scientists identified the seeds which not belonging to the grass family as a pseudo-cereal seeds, Quinoa (*Chenopodium quinoa*) is one of a pseudo-cereal, grown in South America (Andean region). The Food and Agriculture Organization of the United Nations (FAO) launched the year 2013, the “international year of quinoa”, as a step in the promotion of planting, harvesting, production, preservation, and consumption of this seeds. Furthermore, quinoa has been recognized as a nutritious cereal (Vilcacundo and Hernández-Ledesma, 2017).

In a nutritional studies by Fischer et al. (2017) revealed that the quinoa seeds contain high protein with a bioavailable essential amino acids, dietary fibers, complex carbohydrate, lipids with high amount of unsaturated fatty acids, and other bioactive compounds *i.e.* polyphenols (flavonoids, lignins, stilbenes, tannins...
and phenolic acids). Moreover, quinoa seeds are an excellent ingredient in healthy and tasty foods. The easy digestibility of quinoa seeds and gluten-free are attributed these seeds as an unusually complete food because they have a well-balanced set of essential amino acids for humans, as well as being a good source of protein, fiber, phosphors, magnesium, and iron.

Recent studies have identified quinoa seeds as a good source of bioactive-polyphenols which might change antioxidant status in the organism and prevent oxidative stress. And, because of that, Phenolic compounds are considered to carry many potential beneficial health effects (Repo-Carrasco-Valencia and Serna, 2011).

The opportunity to supplement or completely replace common cereal grains (corn, rice and wheat) with a pseudo-cereal of higher nutritional value (such as quinoa) is inherently beneficial to the public interests. Unfortunately, quinoa seeds contain bitter-tasting constituents (chiefly water-soluble saponins) located in the outer layers of the seed coat, making it essentially unpalatable. Therefore, most commercial quinoa seeds, have been processed to remove their coating by washing or milling so to eliminate bitter compounds before consumption (Popenoe et al., 1989).

Triterpenoid saponins are a diverse group of compounds characterized by the presence of a triterpenoid aglycone backbone (C₃₀H₄8O) with one or more sugar moieties combined to them with ester or glycosidic bonds (Kakhimov et al., 2016). Saponins have a astringent taste and bitterness due to their chemical structure. The presence of saponin in food products is not desired and should be reduced to decrease bitterness (Gómez-Caravaca et al., 2014).

For many years ago, irradiation processing was well considered as physical and non-thermal or cold method to preserve foods and food products by exposure of foods to ionizing radiation (Antonio et al., 2012). In study by Behgar et al. (2011) showed that, irradiation may influence the levels of antioxidant phytochemicals either with increased or decreased levels of these plant phytochemicals therefore, gamma irradiation may maintain the antioxidant activity of several plant materials.

In addition, Harrison and Were (2007) found that gamma irradiation increased the phenolic content in almond skin as well as, cinnamon and clove while, the content of phenolic compounds unchanged in nutmeg. On the contrary, other studies by Ahn et al. (2005) revealed that low and medium doses usually have insignificant influence on phytochemical antioxidants where, up to 10 kGy did not have any effect on the antioxidants activity of anise, cinnamon, mint, licorice, nutmeg vanilla and ginger.

Consequently, the employment of quinoa after removal/ reducing of saponin compounds can represent a promising choice leading to additional advantages. In this context, our work aimed to subject quinoa flour to gamma irradiation in order to study the effect of irradiation on the saponin concentration. As well as, study the effect of gamma irradiation on phenolic and flavonoid compounds and antioxidant capacity of quinoa.

2. Material and Methods

2.1. Materials

Simple samples of quinoa (Chenopodium quinoa) seeds freshly packaged were purchased from Egyptian local health-food store. Quercetin, Folin-Ciocalteu reagent, iron (III) chloride, aluminium chloride and sodium bicarbonate were purchased from Sigma (St. Louis, MO, USA). 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium acetate trihydrate, glacial acetic acid, (2, 4, 6-tripryridyl-s- triazine, TPTZ), hydrogen chloride, Ferric chloride hexahydrate (FeCl₃, 6H₂O) and Ferrous sulfate heptahydrate (FeSO₄, 7H₂O) were purchased from Sigma-Aldrich, Germany. All used chemicals were analytical grade.

3. Methods

3.1. Irradiation treatment

Fine powder of de-hulled quinoa seeds were irradiated with gamma irradiation at doses of 3 and 6 kGy using an experimental 60Co Gamma chamber (dose rate 665.6 Gy/h), Cyclotron Project, Nuclear Research Center, Atomic Energy Authority, Egypt.

3.2. Preparation of quinoa extract

Quinoa seeds were extracted using the procedure of Carciochi et al. (2014) with some modifications. Two gram of quinoa flour samples was extracted with 20 mL of 80% ethanol. The mixture was kept in agitation for 30 min at 160 rpm in an orbital shaker. Then, the homogenate was centrifuged for 10 min / 5 °C at 3500 g (VS-550, Vision Scientific Co., Ltd., Bucheon Korea) the clarified extract was collected and then subjected to rotary evaporator at 40 °C under reduced pressure to remove solvent. Finally, the extract was stored in brown bottle and stored at -18 °C for further analysis.

3.3. Determination of Polyphenolic content

3.4. Determination of Total Phenolic Content (TPC)

Total phenolic content of the extracts of both irradiated and non-irradiated quinoa seeds was determined by the Folin–Ciocalteu micro-method (Slinkard and Singleton, 1977). Briefly, 20 μl of extract solution were mixed with 1.16 mL distilled water and 100 μl of Folin–Ciocalteu reagent, followed by addition of 300 μl of Na₂CO₃ solution (20% after 1 min and before 8 min. Subsequently, the mixture was incubated in a shaking incubator at 40 °C for 30 min and its absorbance was measured at 760 nm. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams / 100 g dry material.

3.5. Determination of Total Flavonoid Content (TFC)

Total flavonoid content (TFC) of the extracts of both irradiated and non-irradiated quinoa seeds was determined using the aluminium chloride (AlCl₃) method according to a reliable approach using quercetin as the standard (Ordonez et al., 2006). In this regard, the plant extract
(0.1 mL) was added to 0.3 mL of distilled water followed by addition of 0.03 mL of NaNO$_3$ (5% w/v). After 5 min. at 25 °C, AlCl$_3$ (0.03 mL, 10%) was added. After further 5 min., the reaction mixture was treated with 0.2 mL of Na OH (1 mM). Finally, the reaction mixture was diluted to 1 mL with water and the absorbance was measured at 510 nm. The results were expressed as quercetin equivalents (QE) in milligram / 100g of dry material.

3.6. Chromatographic analysis of polyphenols compounds

High Performance Liquid Chromatography (HPLC) analysis was carried out using an Agilent 1260 series. The separation was carried out using Eclipse Plus C18 column (4.6 mm x 250 mm i.d., 5 μm). The mobile phase consisted of water (A) and 0.02% tri-floro-acetic acid in acetonitrile (B) at a flow rate 1 mL/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (80% A); 0 - 5 min (80% A); 5 - 8 min (40% A); 8 - 12 min (50% A); 12 - 14 min (80% A) and 14 - 16 min (80% A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 10 μl for each of the sample solutions. The column temperature was maintained at 35 °C.

3.7. Determination of foaming and foaming index (FI)

Detection of saponins by foaming test was according to Kokate (1999) with some modifications by Mir et al. (2016). About 2 g of the powdered sample was boiled in 20 mL of distilled water in a water bath and filtered. 10 mL of the filtrate was mixed with 5 mL of distilled water and shaken vigorously for a stable persistent froth. A two cm layer of foam indicated the presence of saponins. Foaming index is measured according to methods described by Kozioł (1990a) using the following Equation 1:

\[
\text{Foamin Index (FI)} = \frac{1000}{a}
\]

Where: a the volume in mL of the decoction used for preparing the dilution in the tube where foaming to a height of 1 cm is observed.

3.8. Determination of Saponin Percentage

The saponin content in the samples was evaluated by a semi-quantitative method described by Kozioł (1990a). This method is based on the tensioactive properties of the saponins, which form a stable foam whose height is related to the saponin content in the grains after being dissolved in water and shaken. The percentage of saponins was obtained with following Equation 2:

\[
\text{Saponin} (\%) = \frac{0.646 x \text{(height of foam, cm)} - 0.104}{\text{(weight of the sample, g)} x 10}
\]

3.9. Determination of Antioxidant Activity

3.9.1. Free radical scavenging activity (DPPH• test)

The DPPH method of Lee et al. (2003) was used with some modifications. The stock reagent solution (1x 10$^{-3}$ mol/ L) was prepared by dissolving 22 mg of DPPH in 50 mL of methanol and stored at – 20 °C until use. quinoa seeds extract (0.1, 0.2, 0.4 and 0.8 mg/mL) and synthetic antioxidant Butylated Hydroxy Toluene (BHT) solution (0.2 mg/mL) (0.1 mL of each) were vortexed for 30 s with 3.9 mL of DPPH• solution and left to react for 30 min, after which the absorbance at 517 nm was recorded. Radical scavenging activity is the method in which the color of the DPPH solution changing from purple to yellow as an indicator of antioxidant activity by radical quenching (Karagözler et al., 2008). A control with no added extract was also analyzed. Results were expressed as a percentage DPPH• radical scavenging activity of a sample and were calculated according to the following Equation 3:

\[
\text{DPPH radical scavenging activity (\%) = } \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100 \quad (3)
\]

Where, \(\text{Abs control}\) = the absorbance of DPPH radical + methanol; \(\text{Abs sample}\) = the absorbance of DPPH radical + samples.

The analyses were done in triplicates and the concentration of extract proportional to a 50% inhibition of DPPH radical (IC$_{50}$) was obtained through the analysis of the extract solution concentration versus inhibition percentage graphic. Thus, lower extract concentrations (μg / mL, ppm) mean greater antioxidant capacity.

3.10. Ferric reducing antioxidant power (FRAP) assay

3.10.1. Reagents preparation

a) Acetate buffer 300 mM pH 3.6: Weigh 3.1g sodium acetate trihydrate and add 16 mL of glacial acetic acid and make the volume to 1 L with distilled water. b) TPTZ (2, 4, 6-tripyridyl-s- triazine), 10 mM in 40 mM HCl, c) FeCl$_3$, 6 H$_2$O, 20 mM. The working FRAP reagent was prepared by mixing a, b, and c in the ratio of (10: 1: 1) just before testing. Standard was FeSO$_4$, 7 H$_2$O: 0.1 - 1.5 mM in methanol.

3.11. Analytical Procedures

FRAP solution (3.6 mL) add to distilled water (0.4 mL) and incubated at 37°C for 5 min. Then this solution mixed with certain concentration of the quinoa extract (80 mL) and incubated at 37 °C for 10 min. The absorbance of the reaction mixture was measured at 593 nm. For construction of the calibration curve, five concentrations of FeSO$_4$, 7H$_2$O (0.1, 0.4, 0.8, 1, 1.12, 1.5 mM) were used and the absorbance values were measured as for sample solutions (Al-Farsi et al., 2005).

3.12. Statistical analyses

All the experiments were carried out in triplicate and mean and standard error were calculated for all data. Then the results were subjected to one-way analysis of variance followed by Duncan’s significant differences using SAS program (version 9.1.3) software (Cary, NC). Significant levels were defined as (P < 0.05) (Statistical Analysis System, 2004).
4. Results and Discussion

4.1. Total phenolic and flavonoid content

Polyphenols are bioactive secondary plant metabolites that are widely present in commonly consumed foods of plant origin. The main types of polyphenols are flavonoids, and tannins, which act as powerful antioxidants in vitro. Typical phenolics that possess antioxidant activity are known to be mainly phenolic acids and flavonoids. TPC and TFC of quinoa flours were given in Figure 1. As regard, the flavonoid content in all samples either irradiated or non-irradiated was higher than the phenolic content. Similar findings were obtained by Pellegrini et al. (2018) who found that the total flavonoid and phenolic content were 490.2 and 2239 mg/kg, respectively in quinoa seeds.

Our present study revealed that TPC were 26.25, 34.52 and 30.92 mg GAE/100g in samples at doses 0, 3 and 6 kGy. These levels tended to be higher than those reported by Park et al. (2017) but lower than Carciochi et al. (2014), Diaz-Valencia et al. (2018), Nsimba et al. (2008), and Pellegrini et al. (2018). On the other hand, TFC were 53.15, 67.44 and 62.89 mg QE/100 g in samples at doses 0, 3, and 6 kGy, the preceding data were higher than those obtained by Carciochi et al. (2014) and Park et al. (2017). These differences might result from the different standards and solvents used in extraction.

The Folin–Ciocalteu procedure is the common method for phenolic content determination, this method does not give quality and quantity of the phenolic compounds in the extracts, in a full picture. However, although all flavonoids are polyphenols, polyphenols not necessarily are flavonoids. As well as, there are many factors affect the polyphenolic compounds (flavonoids and phenols) formation i.e.; genotypes (cultivar and variety), plant maturity, environmental conditions, harvest, and post-harvest conditions (Hardman, 2014).

As shown in Table 1 the polyphenols (TPC and TFC) were varied from irradiated and non-irradiated, i.e.; gallic acid slightly increased at dose 6 kGy. Our data are in agreement with Kumari et al. (2009) who study the effect of irradiation treatment on the antioxidant activity of Triphala, the gallic acid concentration and total phenolics in water extracts were increased after irradiation thus, antioxidant activity was increased. Catechin was increased from 29.311 mg/100g in non-irradiated sample to 32.89 and 37.94 mg/100g in 3 and 6 kGy, respectively.

Some polyphenols were totally disappeared after irradiation treatment like; rutin, coumaric acid, vanillin, sinapic, rosmarinic acid, 4’,7-DihydroxyisoFlavone and apigenin. These changes in profiling analyses in all samples might be due to exposure to gamma irradiation alter/convert some chemical compounds into another ones as well as, the increased phenolic content due to irradiation treatment could be attributed to the release of phenolic compounds from a glycosidic component and the degradation of larger phenolic compounds into smaller ones by gamma radiation.
This point of view, supported by Variyar et al. (2004) who demonstrated that gamma-ray doses of 0.5 - 5 kGy transformed genistein, an isoflavone glycoside, into its aglycon genistin with increasing radiation dose. As well as, Breitfellner et al. (2002) reported that degradation of phenolic acids (gallic, ρ-coumaric, cinnamic and hydroxybenzoic acids) was observed after gamma irradiation at doses from 1 to 10 kGy of strawberries. Different hypothesis was assumed to that degradation one of them, the formation of free hydroxyl (-OH•) radicals caused decomposition/hydroxylation to these phenolic acids.

### 4.2. Total saponin content

Different quinoa varieties are classified according to their content of saponin, in regard to saponin concentration, quinoa may be distinguished in; when saponin concentration was 0 – 0.11% “sweet quinoa” and when saponin concentration is higher than 0.11% “bitter quinoa” (Martinez et al., 2009).

As shown in Figure 2 saponin content was 787 mg/kg while the content it was 755 and 701 mg/kg in quinoa subjected to 3 and 6 kGy, respectively. All samples in our study classified on the basis of saponin content present as “sweet” quinoa.

Irradiation treatment showed slight differences in the saponin content after exposure to 3 and 6 kGy. These results are completely deferent with Mohamed (2009) who mentioned that the saponin content of Eryngium foetidum L. was increased as gamma irradiation dose increased up to 40 Gy. While the results are agreed with Na and Lee (2010) when found red ginseng saponins very stable to gamma irradiation.

The most important property of saponin is soap-like or detergent and cause persistent foam when an aqueous decoction is shaken. The foaming ability of an aqueous decoction of herbal materials and their extracts is measured in terms of a foaming index (Shirsat et al., 2017). The foaming index method is the only standard method for quantitative determination of saponin content in plant materials as reported by Kozioł (1990a).

However, our present data also revealed that foaming index in non-irradiated samples (125) where higher than that in irradiated samples (111.1) at both irradiated doses 3 and 6 kGy.

### 4.3. Antioxidant activity of quinoa seed extract

#### 4.3.1. DPPH• Radical scavenging activity

The antioxidant activity was measured in regard to the radical scavenging, according to the DPPH method (Table, 2). At gamma irradiation dose 3 kGy the DPPH-scavenging activity (0.1 to 0.4 mg/mL) was 33, 47.5, 57.3 and 65.3 mg/mL compared to non-irradiated 29.3, 40.6, 55.2 and 62.4 mg/mL, respectively. Thus, the irradiation dose up to 3 kGy showed significantly (p > 0.05) increased in DPPH capacity. Gamma irradiation enhanced reactive scavenging capacity of soybean extracts as reported by
Variaty et al. (2004). While 6 kGy significantly (p > 0.05) decreased the scavenging activity.

The same finding was observed by Moosavi et al. (2014) who found that 2kGy slightly increased radical scavenging activity of almond hull and the lowest values of scavenging activity was observed at 10 kGy. Antioxidant content of fresh plant is either increased or decreased due to irradiation treatment, and the radiation is dependent on exposure time, raw material and dose absorbed (Bhat et al., 2007).

Our obtained data are similar to Brand-Williams et al. (1995) in the higher levels of DPPH antiradical activity in samples that were subjected to irradiation treatment correlated with higher levels of phenolic and flavonoid. Most of the plant extracts which have antioxidant activity is related to its content of different phenolic compounds.

These compounds are commonly contain at least one hydroxyl substituted aromatic ring system, that can easily oxidized, in addition serving as important units for donating ability on DPPH to produce DPPH-H, considered as an essential mechanism of antioxidants. Moreover, irradiation especially at low doses results in the intracellular generation of hydrogen peroxide and reactive oxygen species (ROS), which may alter the plant antioxidant content (Kovacs and Keresztes, 2002).

Antioxidant capacity can be measured as inhibitory concentration 50 (IC_{50}), which is the concentration required to get 50% antioxidant capacity measured as DPPH. The lower the IC_{50} values the higher the radical scavenging activity gained. Figure 3 showed the IC_{50} of irradiated and non-irradiated quinoa. Three kGy represented the lowest IC_{50} (0.217 mg/mL) compared to both non-irradiated (0.24 mg/mL) and quinoa applied to 6 kGy (0.282 mg/mL).

Figure 3. Radical Scavenging activity (DPPH IC_{50}, mg/mL) of irradiated and non-irradiated quinoa.

Nsimba et al. (2008) who worked on different ecotypes of quinoa, from Japan and Bolivia highlands, presented IC_{50} values between 100 – 7500 μg/mL (ecotypes of Japan) and 300-15800 μg/mL (ecotypes of Bolivia Altiplano). Alvarez-Jubete et al. (2010b) and Nsimba et al. (2008) mentioned that the antioxidant activity of quinoa is varied according to differences of its ecotypes, where the presence of phenolic compounds was influenced by different factors i.e.; genetic factors, agrotechnical process, and environmental conditions.

4.3.2. Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay depends on the reduction of ferric TPTZ [Fe (III)-TPTZ] to ferrous TPTZ [Fe (II)-TPTZ], which has an intensive blue color and can be monitored at 593 nm, by a reductant either reducing agents or antioxidants, at low pH (Xu and Chang, 2007).

The FRAP values for the quinoa seed extract are presented in Table 2. The quinoa seed extract exhibited the highest FRAP value with 6.29 mM Fe^{2+}/kg dry material at gamma irradiated dose 3 kGy, followed by 6 kGy (5.86 mM Fe^{2+}/kg dry material), and non-irradiated quinoa (5.49 mM Fe^{2+}/kg dry material). Furthermore, all FRAP data in our present study increased in irradiated quinoa compared to non-irradiated. The FRAP values of quinoa seed extracts were closer to the values previously reported by Nsimba et al. (2008), whereas the FRAP value of quinoa seed extract in the present study was higher than obtained by Brend et al. (2012). Furthermore, the obtained results illustrated that there was a correlation between phenolic contents and ferric reducing antioxidant power. This finding declared that the importance of phenolic acids content as a reducing agent in our study and this may be due to their potent electron donating abilities (Bilto et al., 2012).

Radiation treatments have been shown to either increase or decrease the antioxidant content of fresh plant produce, which is dependent on the dose delivered, exposure time, and the raw material used. The enhanced antioxidant capacity/activity (correlation with the total phenolic content accordingly antibacterial activity) of a plant after irradiation is mainly attributed either to increase enzyme activity (e.g., phenylalanine ammonialyase and peroxidase activity) or to the increased extractability from the tissues (Bhat et al., 2007).

In addition, Khattak et al. (2008) mentioned that the antioxidant activity was either increased or decreased of

| Table 2. Antioxidant activity of irradiated and non-irradiated quinoa. |
|---------------|---------------|---------------|---------------|---------------|---------------|
| **FRAP (mM Fe^{2+} / kg, dm)** | **DPPH (mg/mL)** |
|            | 0.1           | 0.2           | 0.3           | 0.4           |
| 0 kGy      | 5.49 ± 0.31\(^a\) | 29.3 ± 0.51\(^a\) | 40.6 ± 0.37\(^b\) | 55.2 ± 0.49\(^a\) | 62.4 ± 0.73\(^a\) |
| 3 kGy      | 6.29 ± 0.27\(^a\) | 33.0 ± 2.32\(^a\) | 47.5 ± 0.43\(^b\) | 57.3 ± 0.65\(^a\) | 65.3 ± 0.89\(^a\) |
| 6 kGy      | 5.86 ± 0.07\(^a\) | 30.1 ± 1.01\(^a\) | 39.6 ± 0.50\(^b\) | 42.5 ± 0.98\(^b\) | 53.8 ± 0.94\(^a\) |

Each value represents the mean ± S.E., the mean value with different superscript alphabets in the same column indicate significant differences (P<0.05) using Duncan test, FRAP (ferric reducing antioxidant power), dm (dry mater), DPPH (1,1-diphenyl-2-picrylhydrazyl).
irradiated samples might be due to the different of solvents used for extraction. However, the scavenging activity was enhanced after irradiation treatment.

5. Conclusion
Quinoa seeds contain high amount of polyphenols and tends to have potent antioxidant capacity. In general, gamma irradiation changed the phytochemical content and antioxidant activity of quinoa seeds flour. 3 kGy was the enhancement dose to maintain antioxidant content and activity. Regarding the saponin, it tends to be resistance to many processes and so gamma irradiation and calls for further investigations.

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