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Biochemical Impacts of Radiation Processing on Grapes and Artichoke Agro Industrial Wastes

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Authors' contributions

This work was carried out in collaboration between all authors. Authors DMS and DEHF designed the study, wrote the protocol, managed the analyses of the study supervised the work and wrote the first draft of the manuscript. Authors NSH and MAE managed the literature researches, the analyses of the study and edited the manuscript. Author AMM carried some laboratories work and performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Food irradiation is a method of preservation used to extend the shelf life of food products fresh and/or dried, destroy the contaminating harmful pathogens and modify the activity of bioactive compounds present in food materials.
Objective: The present study was conducted to test the possible biochemical impacts of radiation processing on wastes of grapes and artichoke, and to elucidate the physiological and biochemical effects of feeding growing male Wistar rats on diets supplemented with processed agro industrial wastes at dose levels of 10, 20 and 30 kGy.
Results: No significant differences were detected in the chemical composition of processed agro industrial wastes at all doses levels compared to non-irradiated one. Irradiation processing caused

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different changes in polyphenols and tannin contents as a function of radiation dose. Regarding the impact of irradiation treatment, up to 30 kGy on free radical scavenging activity of grape seeds-skin, and artichoke leaves, the results showed that there was a significant reduction in their ability to scavenge free radicals, while an exception was observed for artichoke leaves processed by 10 kGy. Changes in amino acids and fatty acids patterns were observed, as affected by irradiation (10- 30 kGy), without a specific trend in the two agro industrial wastes under investigation. The physiological and biochemical performance of growing male Wistar rats affected by feeding high fat diets supplemented with non-irradiated or irradiated agro industrial wastes under investigation for 8 weeks, showed better results when compared with those fed on reference diet. It has been revealed in body weight gain and internal organ weight alongside biochemical aspects such as serum lipid profile, AI, ALT, AST and plasma glucose.

Conclusion: No physiological and biochemical critical changes were observed on male Wistar rats due to irradiation. Further researches are needed to estimate the effect of radiation processing on bioactive compounds of the studied agro-industrial wastes extract.

Keywords: Grape seed-skin; artichoke leaves; γ-irradiation; antioxidants.

1. INTRODUCTION

During endogenous metabolic reactions, aerobic cells produce reactive oxygen species (ROS) such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), and organic peroxides as normal products of the biological reduction of molecular oxygen. These ROS have several important physiological functions, but their accumulation beyond the needs of the cell can potentially damage lipids, proteins, and nucleic acids [1]. Presence of excess ROS cause oxidative stress. The continued oxidative stress could mediate most chronic diseases, including cancer, diabetes, cardiovascular, neurological, aging and pulmonary diseases [2]. In such conditions, external supply of antioxidants is essential to countervail the deleterious consequences of oxidative stress [3,4].

On the top of that, ROS are strongly associated with lipid oxidation in food leads to a significant loss of its nutritional quality and cause the formation of toxic compounds. For this reason, synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butyhydro-quinone (TBHQ), are widely used in the food industry as potential antioxidants [5]. However, natural antioxidants, of plant origin mainly, might help to attenuate the oxidative processes [6], and have been of great concern [7,8]. The antioxidant capacity of plant foods is derived from the cumulative synergistic action of a wide variety of antioxidants such as vitamin C, E and polyphenols, mainly phenolic acids and flavonoids, carotenoids, terpenoids, maillard compounds and trace minerals [9].

The processing of plant foods results in the production of by-products that are rich sources of bioactive compounds, including phenolic compounds [10,11,12]. Phenolic compounds with antioxidant activity have been identified in several agricultural by-products, such as rice hulls [13], buckwheat hulls [14] and almond hulls [15], and also 26 different fruit and vegetable wastes [16].

Peels of several fruits have been found to contain higher amounts of phenolics than the edible fleshy parts, as peels from apples, peaches and pears [17,18]. Grapes (*Vitis vitaceae*) are members of the family *Vitaceae*. Grape berries are consumed as fruit, wine, juice, and raisins. Grape vines and their products, particularly wine, have been important elements in human life, foods and religions [19].

The benefits of consuming grapes and its products have gained further recognition with the discovery of phenolics. Phenolic compounds were demonstrated to reduce atherosclerosis, coronary heart disease [20,21,22,23], various cancer types, and several dermal disorders [24].

Grape pomaces are the major wastes generated in the wine making process and the utilization of its components, (such as skins, pulp, stalks and seeds), have an important environmental impact on waste reduction and permit the production of added value products [25,26,27,28,29]. The utilization of by-products from wine making is an urged problem in Europe within context of recently posed regulations presuming measures against burying of wine by-products affecting the soil erosion/compaction and the quality of groundwater [30].

Artichoke belongs to *Cynara* genus which is a relatively small genus, originating from the Mediterranean area that includes two types of crops, globe artichoke (*Cynara cardunculus* var. *scolymus L.*) and cardoon (*Cynara cardunculus* var. *altilis DC*) [31,32,33]. These plants were popular in Greeks and Romans as food and medicine [34,35].

Globe artichoke has a low content of fat and high levels of minerals (potassium, sodium, and phosphorus), vitamin C, fibers, polyphenols, flavones, inulin and hydroxycinnamates-caffeoylquinic acid derivatives [36,37,38]. According to Foti et al. [31] the chemical composition of artichoke seeds was as follows: Crude protein 21.6%, crude fiber 17.1%, crude oil 24.05% and ash 3.8%. The artichoke flower heads have a high content of vitamin C (10 mg / 100 g FW) and minerals (K 360 mg / 100 g FW; Ca 50 mg / 100 g FW) [36], where FW is fresh weight. Leaves and heads of artichoke have been found to be rich in polyphenolic compounds, inulin, fiber and minerals [35].

However, plant materials are highly susceptible to microbial contamination due to the medium (water and soil) in which they grow, harvesting and storage. Processing may cause additional contamination and microbial growth [39]. Thus, an adequate technology for decontamination is required to improve the hygienic quality of plant materials and to make it suitable for human use and commercialization [39,40].

Conventional methods of microbial decontamination are fumigation with either gaseous ethylene oxide or methyl bromide, which are carcinogenic and hence are now prohibited according to Montreal protocol, or being increasingly restricted in most advanced countries for health, environmental or occupational safety reasons. The most advanced method used currently to eliminate pathogen from food, feed and agro-industrial wastes are gamma irradiation process. It is well known as a decontamination method for many foodstuffs and plant materials, being an environmentally friendly and effective technology to resolve technical problems in trade and commercialization [41,42].

Ionizing radiations (γ and e-beams) and UV light can inactivate food borne microorganisms without substantially heating the food [43]. The use of γ-irradiation to enhance the shelf life of minimally processed fruits and vegetables and to ensure the microbiological safety is increasing because it appears to be effective both on cells and on spores. On the other hand, food irradiation can be used to reduce insect infestation of grain, dried spices, and dried or fresh fruits and vegetables; inhibit sprouting in tubers and bulbs; retard postharvest ripening of fruits; inactivate parasites in meats and fish; eliminate spoilage microbes from fresh fruits and vegetables; extend the shelf life of poultry, meats, fish, and shellfish; decontaminate poultry and beef; and sterilize foods and feeds [44].

This study aimed to test the possible biochemical impacts of radiation processing on two agro industrial wastes (grape seed-skin and artichoke leaves), in addition to elucidate the physiological and biochemical effects of feeding growing male Wistar rats on diets supplemented with the irradiated agro industrial wastes at dose levels of 10, 20 and 30 kGy.

2. MATERIALS AND METHODS

2.1 Materials

Agro-industrial by-products of Roumy grapes (*Vitis vinifera)* pomace and French Artichoke (*Cynara scolymus)* heads were obtained from Al-Obour central market.

2.1.1 Preparation of samples

Fresh samples were dried in shade under continuous air using electrical fan, at room temperature (25ºC) and grounded to the finest particles.

2.1.2 Radiation processing

Dry grounded samples were packed in well-sealed polyethylene sleeves (1000 gauge, 0.25 mm thickness). They were subjected at room temperature to gamma irradiation at dose levels of 0, 10, 20 and 30 kGy, at dose rate 10 kGy/ 3 hours as monitored by FWT-60-00™ radio chromic film. The radio-chromic dosimeter was purchased from Far West Technology, Inc., Goleta, California, USA, (ASTM, 2002 [ISO/ASTM 51275:2002(E)]). The irradiation facility used was Egypt's Mega Gamma-1 Type J-65000 located at the National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt. The non-irradiated and irradiated samples were stored at 4ºC until used.

2.1.3 Animals

A total number of 60 Wistar male rats with initial weight of 100-120 g were obtained from animal house of National Center for Radiation Research

and Technology, Cairo, Egypt; animals were examined by veterinarian. The animals were healthy and clinically free of internal and external parasites. Animals were kept in the animal house at an ambient temperature 22ºC±3 and 50±5% relative humidity with a 12h each of dark and light cycle and water ad libitum, all groups were adapted for one week under standard ration meets their nutritional requirements before the experiment.

2.1.4 Feeding experiment

Rats were individually weighted and divided equally into 10 groups. Each group was assigned for specific diet, D1: Reference diet (Table 1), D2: Reference diet plus 20% fat, D3: High fat diet incorporated with non-irradiated wastes, D4: High fat diet incorporated with 10 kGy irradiated wastes, D5: High fat diet incorporated with 20 kGy irradiated wastes, D6: High fat diet incorporated with 30 kGy irradiated wastes, rats kept on these diets for 8 weeks. At the end of the experimental period, blood was obtained by cardiac puncture using a 20 gauge needle according to the method reported by Dacie and Lewis [45], and blood samples from each rat were collected in two sterile test tubes, one contain sodium fluoride to estimate plasma glucose, the other containing blood samples and centrifuged at 5000 rpm for 10 minutes, then serum was separated and collected.

2.2 Methods

2.2.1 Chemical composition

Moisture content, crude fat, crude protein, ash content were determined according to the standard methods described in the A.O.A.C. [46], crude fiber was determined according to standard method described in the A.O.A.C [47], and nitrogen free extract (NFE) was calculated according to the equation:

NFE (%) = 100 - (%Protein + %Fat + % Crude Fiber + %Ash + %Moisture)

2.2.2 Lipid extraction and determination of fatty acids methyl esters by GLC

The lipid content of dried ground, non-irradiated and irradiated, grape seeds-skin and artichoke leaves was extracted using diethyl ether. The free fatty acids were methylated by methylating agent according to Hamilton and Hamilton [48]. Fatty acids methyl esters were dissolved in chloroform (HPLC grade) and chromatographic separation was performed using hp 6890 gas chromatography instrument equipped with a flame ionization detector using innowax-cross linked polyethylene glycol fused silica column (30 m long, 0.32 mm i.d. 0.5 µm film thickness). Oven temperature was programmed from 150ºC for 1 min then elevated to 235ºC with a rate of 17ºC/ min then, raised again to 245ºC with a rate of 1ºC/ min and hold at 245ºC for 5 min. Gasses flow rates for N_2 as a carrier gas, H_2 and air were 1.3, 40 and 400 ml/min, respectively. Flame ionization detector and injection temperatures were 275ºC and 260ºC, respectively.

Table 1. Composition of reference diet (maize/soybean) diet

Ingredients	g kg^{-1}
Maize	600
Soybean meal	200
Wheat bran, fine	20
Layer Concentrate, (40% Protein)	116
Sunflower oil	16
Limestone	43
Salt, iodized	2
Mineral premix[1]	1
Vitamin premix[2]	2
Total	1000
Calculated analysis	
Metabolizable energy, MJ kg^{-1}	12.15
Crude protein, g kg^{-1}	215
Available phosphate, g kg^{-1}	4.5
Calcium, g kg^{-1}	25.2
Lysine, g kg^{-1}	10.9
Methionine, g kg^{-1}	4.5
Methionine + Cystine, g kg^{-1}	7.7

[1] Supplied per kg of diet: 12 mg of Cu, 98 mg of Fe, 50 mg of Zn, 80 mg of Mn, and 0.35 mg of I and 0.2 mg of Co.

[2] Supplied per kg of diet: 8000 IU of vitamin A, 200 IU of vitamin D_3, 40 IU of vitamin E, 4.5 mg of vitamin B_1, 9 mg of vitamin B_2, 9 mg of Vitamin B_6, 0.04 mg of vitamin B_{12}, 85 mg of niacin, 27 mg of D-pantothenic acid, 0.4 mg of biotin, and 1.2 mg of folic acid.

2.2.3 Amino acids analysis

A known amount of the defatted sample (50 mg) was hydrolyzed with HCl (5 ml, 6N) and heated in a sealed test tube at 110ºC for 24h according to Suzanna [49]. The contents of each tube were filtered, evaporated until dryness and a suitable volume of sodium citrate buffer (pH 2.2) was added to dissolve the hydrolyzed sample followed by ultra-filtration using a 0.2 µm membrane filter [50]. Analyses were performed using high performance amino acids analyzer (Biochrom 20, Auto sampler version; Pharmacia

Biotech). Detection was performed at two wave lengths (570 and 440 nm). The data of each chromatogram was analyzed by EZ chrome^{TM} chromatography data system tutorial and user guide-version 6.7.

2.2.4 Tannins analysis

It was determined according to, Balbaa [51].

2.2.5 Free radical scavenging ability (DPPH assay)

The antioxidant activity of grape seeds-skin and artichoke leaves methanol extract was determined, based on the radical scavenging ability in reacting with a stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to Blois [52].

2.2.6 Total phenolic compounds

Extraction and determination of phenolic compounds were conducted according to the method described by Daniel and George [53].

2.2.7 Biochemical assays

Serum alanine transaminase (ALT) and aspartate transaminase (AST) were determined according to Sherwin and Pesce [54]. Determination of total serum cholesterol was done according to Richmond **[**55], Serum HDL-Cholesterol [56], serum triglycerides was determined according to Mgowan et al. [57], serum low density lipoprotein cholesterol (LDL-C) was calculated by the equation: Serum LDL-C (mg/dl) = total cholesterol- HDL-C - triglycerides/5 [58], serum very low density lipoprotein cholesterol (VLDL-C) was calculated by the equation: Serum VLDL-C mg/dl = Triglycerides/5 [59]. Atherogenic Index (AI) was calculated using the equation, AI = (total cholesterol-HDL-C)/HDL-C [60], Plasma glucose was measured by the method of Caraway and Watts [61].

2.3 Statistical Analysis

The data were subjected to analysis of variance (ANOVA) with one-way classification. Linear regression analysis was utilized to define the relationship between different parameters and irradiation dose (kGy). All analyses were conducted using the general linear model procedure of the Statistical Analysis System Institute, Inc., (SAS, 2003), where appropriate, treatment means were separated using the Duncan's Multiple Range Test [62]. The α-level for significance was $P \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1 Effects of Radiation Processing on Chemical Composition of Grape Seeds-skin and Artichoke Leaves

Complete chemical composition of irradiated grape seeds-skin in Table 2 showed no significant differences from non-irradiated ones. Results of crude protein, moisture and fat of irradiated artichoke leaves (Table 3), at applied radiation doses used, also showed no significant differences from non-irradiated samples. This can be attributed to the relatively limited amount of water content of the two studied wastes (about 111.2, and 26.0 g kg^{-1}, for grape seeds-skin, and artichoke leaves respectively), so it would not be easy to be radiolyzed by irradiation to produce enough free radicals that could induce significant changes in the gross composition of these materials.

Crude protein and fat in a complex matrix of foodstuffs have been reported to be more resistant to radiation than in the pure state [63]. Crude fiber of artichoke leaves was significantly decreased as radiation dose increased from 10 kGy to 30 kGy. On the other hand, nitrogen free extract (NFE) content was increased as radiation dose increased; these can be attributed to break down of fiber molecules by the absorbed energy leading to the formation of compounds of lower molecular weight. These explanations agree with Hussain et al. [64] the author studied four irradiated bean varieties (red, yellow, black and white), by γ-rays at dose levels 5 to 25 kGy.

The results agree with Ashraf [65], in his study on the effect of processing soybean, broad bean and pea by ionizing irradiations (gamma and electron beam rays) at dose levels 5, 10, 30 and 60 kGy of both ray sources. El-Niely [66]; Ali [67] and Farag [68] reported that irradiation processing didn't cause significant changes in the chemical composition of soybean and broad bean agro industrial wastes at the applied radiation doses.

The results of the present study are in line with the results reported on irradiation effects on different types of food, as for Jojoba meal irradiation up to 75 kGy [69], irradiation processing of cotton seed at 20, 40, 60 and 80 kGy [70], canola meal or cassava meal irradiated

at dose levels of 50, 75 and 100 kGy [71], artichoke by-product irradiation at 75 and 100 kGy [72] and sunflower meal irradiation up to and including 40 kGy.

Regarding the results of total phenols of the studied agro-industrial wastes (grape seeds-skin, and artichoke leaves) shown in Table 4, it was suggested that the irradiated samples exhibited an increase in the content of its polyphenolic compound.

Results of tannins content in Table 4 showed that irradiated samples of grape seeds-skin and artichoke leaves were increased and the most pronounced effect was for grape seeds-skin sample irradiated by 20 and 30 kGy. While, the increase in tannins in irradiated artichoke leaves samples at all radiation doses were almost the same.

Phenolic compounds can range in size from monomers to long-chain polymers such as tannins, and usually exist bound to carbohydrates or as part of repeating subunits of high molecular weight polymers [73]. The decrease or increase in tannins content can be explained by that, irradiation processing may cause polymerization of small molecular weight compound leading to the formation of higher molecular weight or breakdown of large molecular weight leading to the formation of low molecular weight polyphenols [74].

The increase in the total phenolics could be attributed to the release of phenolic compounds from glycosidic components and the degradation of larger phenolic compounds into smaller ones by γ- irradiation (Harrison and Were, [73]).

The decrease in tannins of grape seeds-skin irradiated at dose level 10 kGy, agrees with previous studies using γ- irradiation, [75,76] on different types of legumes, while the increase of tannin content in artichoke at all dose levels used agrees with Stajner et al. [77]. The author used soybean seeds and γ- irradiation at dose between 1 to10 kGy.

The opposite effects of irradiation processing were attributed to the different phenolic compounds present in the various plant materials, some have appreciable amounts of hydrolysable tannins as clove, which may be more susceptible to γ-irradiation compared to the condensed tannins present in cinnamon and other spices [73].

In most studies using ionizing irradiation treatment of foodstuffs, a decrease in the level of

Table 2. Chemical composition of raw and gamma irradiated grape seeds-skin

Component (g. kg^{-1})	Dose kGy				Pooled SE	P-value
	0	10	20	30		
Proteins[1]	121.9^{a}±1.2	117.6^{a}±0.66	118.2^{a}±1.50	121.1^{a}±0.75	0.1117	0.1232
Fiber[2]	153.7^{b}±3.38	169.3^{a}±2.96	168.3^{a}±0.33	158.0^{b}±2.73	2.6352	0.0073
Ash[3]	29.9^{a}±1.27	31.2^{a}±0.93	28.7^{a}±1.64	29.6^{a}±0.67	1.1836	0.5118
Fat[4]	124.0^{a}±4.00	132.3^{a}±1.45	124.3^{a}±4.25	133.2^{a}±2.31	0.2502	0.1040
Moisture[5]	111.2^{a}±2.62	108.0^{a}±1.59	111.7^{a}±1.29	106.3^{a}±1.65	1.8558	0.2039
Nitrogen free extract[6]	462.3^{a}±12.37	441.7^{a}±3.73	451.6^{a}±6.11	450.9^{a}±2.57	7.2653	0.3268

Values are means ± SE, Means in the same Raw with the same letter are not significantly different.
[1]Linear (P=0.6959), Quaid (P=0.0322), cubic (P=0.6177), [2]Linear (P=0.3021), Quaid (P=0.0012), cubic (P=0.5336), [3]Linear (P=0.5386), Quaid (P=0.8063), cubic (P=0.1925), [4]Linear (P=0.1680), Quaid (P=0.9113), cubic (P=0.0367), [5]Linear (P=0.2204), Quaid (P=0.5755), cubic (P=0.0917), [6]Linear (P=0.4783), Quaid (P=0.2080), cubic (P=0.2408).

Table 3. Chemical composition of raw and gamma irradiated artichoke leaves

Component (g kg^{-1})	Dose (kGy)				Pooled SE	P-value
	0	10	20	30		
Proteins[1]	208.3^{a}±1.3	198.5^{a}±1.3	200.7^{a}±5.5	204.5^{a}±4.6	3.7127	0.3216
Fiber[2]	534.3^{a}±2.9	517.7^{b}±1.5	516.0^{b}±3.1	461.7^{c}±6.0	3.7527	0.0001
Ash[3]	60.0^{a}±1.2	61.4^{a}±2.3	59.1^{a}±2.0	65.4^{a}±1.8	1.8644	0.1624
Fat[4]	11.6^{a}±0.1	11.2^{b}±0.0	10.5^{c}±0.2	10.5^{c}±0.0	0.1213	0.0005
Moisture[5]	26.0^{b}±0.0	29.7^{a}±0.2	27.6^{b}±0.3	30.0^{a}±1.1	0.5577	0.003
Nitrogen free extract[6]	159.7^{c}±2.3	181.6^{b}±3.2	186.1^{b}±1.2	227.9^{a}±9.4	5.1490	0.0001

Values are means ± SE, Means in the same Raw with the same letter are not significantly different, [1]Linear (P=0.5894), Quaid (P=0.1030), Cubic (P=0.5510), [2]Linear (p=0.0001), Quaid (P=0.0010), Cubic (P=0.0038), [3]Linear (P=0.1333), Quaid (P=0.2224), Cubic (P=0.1816), [4]Linear (p=0.0001), Quaid (P=0.0907), Cubic (P=0.1237), [5]Linear (P=0.0040), Quaid (P=0.2891), Cubic (P=0.0035), [6]Linear (p=0.0001), Quaid (P=0.0898), Cubic (P=0.0450).

tannin was accompanied with an increase in total phenolic compounds [73,77,78,79].

Irradiation processing caused a decrease in DPPH scavenging activity in all processed samples except in artichoke leaves irradiated by 10kGy γ-rays (Table 4). The decrease in the DPPH scavenging activity of irradiated grape seed-skin and artichoke leaves can be neglected, these effects can be explained by the breakdown of ascorbic acid (Vit. C), which is the most sensitive vitamin to irradiation processing [80,81].

3.2 Effect of γ-irradiation Processing on Amino Acid Analysis of Grape Seeds-skin and Artichoke Leaves

All the tested amino acids and calculated parameters: Total amino acids (TAA), total essential amino acids (TEAA), total non-essential amino acids (TNEAA), essential amino acids index (EAAI) and biological value (BV) of γ-irradiated grape seeds-skin at dose level 10 kGy were increased except alanine and cystine (unchanged). Most tested amino acids of γ-irradiated grape seeds-skin at dose level 20 kGy were unchanged, although glutamic, cysteine, TNEAA , EAAI and BV were decreased, while tyrosine, phenylalanine, histidine, cystine, TAA and TEAA were increased (Table 5).

For γ-irradiated grape seeds-skin at dose level 30 kGy, most tested amino acids and calculated parameters were unchanged. Alanine, cystine, histidine, lysine, arginine, TAA, TEAA, TNEAA, EAAI and BV were decreased, while aspartic, threonine and proline were increased.

All tested amino acids and calculated parameters of artichoke leaves irradiated at dose level 10 kGy were decreased. The same effect was observed for samples processed at dose level 20 kGy of γ-rays except for valine, methionine, isoleucine, leucine and TEAA their values were increased, where values of glycine and alanine showed no change from those corresponding values in raw samples. Irradiation of artichoke leaves at dose level 30 kGy caused an increase of most tested amino acids and calculated parameters, with an exception for proline and cystine. Glycine, methionine, tyrosine and lysine showed no change from values of the same amino acids obtained from non-irradiated artichoke leaves (Table 6).

The effect of irradiation processing on amino acid content of agro-industrial wastes under investigation using calculated parameters revealed that TAA in most irradiated samples were increased than non-irradiated ones except grape seed-skin irradiated by 30 kGy, and artichoke leaves irradiated by 10 and 20 kGy TEAA were increased in most irradiated samples except for grape seeds-skin sample irradiated by 30 kGy and artichoke leaves irradiated by 10 kGy. TNEAA were decreased in most samples except for grape seed-skin irradiated by 10 kGy, and artichoke leaves irradiated by 30 kGy were increased. EAAI in most irradiated samples was increased than non-irradiated ones except grape seed-skin irradiated by 20 and 30 kGy and artichoke leaves irradiated by 10 and 20 kGy. Biological value (BV) in most irradiated samples was increased except grape seed-skin irradiated by 20 and 30 kGy, and artichoke leaves irradiated by 10 and 20 kGy.

The general effect of γ-irradiation processing on amino acid analysis can be summarized in that, sulfur-containing acids and aromatic acids are the most sensitive to irradiation, while simple amino acids could be formed by the destruction of complicated amino acids [82].

Changes in the concentration of amino acids induced by irradiation may probably be due to free radicals that might be formed in association with splitting of the peptide bonds [82]. Where, simple amino acids due to irradiation undergo reductive deamination and decarboxylation, aliphatic amino acid with increasing chain length, providing additional C-H bond for interaction with OH- radical reduces the amounts of oxidative deamination [83],the presence of thiol (S–H) or disulfide (S–S) groups in amino acids structure, oxidation of the sulfur occurs, and in aromatic and heterocyclic amino acids hydroxylation of aromatic ring is the principal reaction [84]. Fu et al. [85] mentioned, that radiation-induced free radicals which in turn react on carbon of peptide bonds to induce cleavage of the peptide and cross linkage thus increasing the concentration of the amino acid.

Results of fatty acid analysis in Table 7 showed that palmetic acid was the predominant saturated fatty acid in the non-irradiated wastes and oleic was the predominant unsaturated fatty acid in both grape seed-skin and artichoke leaves.

Table 4. Antioxidant content and activity of raw and gamma irradiated grape seeds-skin and artichoke leaves

	Antioxidant parameter	Dose (kGy)				Pooled SE	P-value
		0	10	20	30		
Grape seeds-skin	Total Phenolic Compounds[1] (mg. g^{-1})	0.78^{c}±0.002	0.8^{b}±0.001	0.66^{d}±0.002	0.81^{a}±0.002	0.0008	0.0001
	Change (%)		(+2.56)	(-15.38)	(+3.85)		
	Tannins[2](mg. g^{-1})	0.04^{c}±0.001	0.03^{c}±0.003	0.049^{b}±0.001	0.05^{a}±0.002	0.0005	0.0001
	Change (%)		(-25.00)	(+22.50)	(+25.00)		
	DPPH[3] (%)	58.22^{a}±0.02	57.52^{b}±0.33	56.87^{c}±0.00	55.74^{d}±0.03	0.0164	0.0001
	Change (%)		(-1.20)	(-2.32)	(-4.26)		
Artichoke leaves	Total phenolic compounds[1] (mg. g^{-1})	0.75^{d}±0.001	0.84^{a}±0.001	0.78^{c}±0.002	0.83^{b}±0.002	0.001	0.0001
	Change (%)		(+12.00)	(+4.00)	(+10.67)		
	Tannins[2](mg g^{-1})	0.08^{c}±0.002	0.09^{b}±0.001	0.09^{b}±0.002	0.09^{a}±0.003	0.001	0.0001
	Change (%)		(+12.50)	(+12.50)	(+12.50)		
	DPPH[3] (%)	48.99^{b}±0.08	50.16^{a}±0.01	47.19^{c}±0.04	46.15^{d}±0.08	0.6	0.0001
	Change (%)		(+2.39)	(-3.67)	(-5.80)		

Values are means ± SE.

Means in the same Raw with the same letter are not significantly different.

Grape seeds-skin

[1]Linear (P=0.0001), Quaid (P=0.0001), cubic (P=0.0001).

[2]Linear (P=0.0001), Quaid (P=0.0001), cubic (P=0.0018).

[3]Linear (P=0.0001), Quaid (P=0.2326), cubic (P=0.4955).

Artichoke leaves

[1]Linear (p=0.0001), Quaid (p=0.0001), Cubic (p=0.0001).

[2]Linear (p=0.0001), Quaid (P=0.0011), Cubic (P=0.0002).

[3]Linear (p=0.0001), Quaid (p=0.0001), Cubic (p=0.0001).

Table 5. Amino acids composition of irradiated and non-irradiated grape seed- skin

Amino acid	Control	10 KGy		20 KGy		30 KGy	
	g/100 g	g/100 g	% Change	g/100 g	% Change	g/100 g	% Change
Aspartic	0.54	0.66	+22.22	0.54	0.00	0.6	+11.11
Threonine	0.42	0.54	+28.57	0.42	0.00	0.48	+14.29
serine	0.48	0.54	+12.50	0.48	0.00	0.48	0.00
Glutamic	0.78	0.84	+7.69	0.72	-7.69	0.78	0.00
Proline	0.12	0.18	+50.00	0.12	0.00	0.13	+8.33
Glycine	0.48	0.54	+12.50	0.48	0.00	0.48	0.00
Alanine	0.36	0.36	0.00	0.36	0.00	0.3	-16.67
Cystine	0.18	0.18	0.00	0.12	-33.33	0.13	-27.78
Valine	0.54	0.6	+11.11	0.54	0.00	0.54	0.00
Methionine	ND	ND	-	ND	-	ND	-
Isoleucine	0.48	0.6	+25.00	0.48	0.00	0.48	0.00
Leucine	1.86	2.04	+9.68	1.86	0.00	1.86	0.00
Tyrosine	0.13	0.24	+84.62	0.24	+84.62	0.13	0.00
Phenylalanine	0.48	0.6	+25.00	0.54	+12.50	0.48	0.00
Histidine	0.72	0.84	+16.67	0.78	+8.33	0.66	-8.33
Lysine	0.9	0.96	+6.67	0.9	0.00	0.78	-13.33
Arginine	1.26	1.32	+4.76	1.32	+4.76	1.14	-9.52
TAA	9.9	11.16	+12.73	10.02	+1.21	9.54	-3.64
TEAA	6.66	7.44	+11.71	6.84	+2.70	6.42	-3.60
TNEAA	3.07	3.54	+15.31	3.06	-0.33	3.03	-1.30
EAAI	10.43	11.82	+13.33	10.24	-1.82	9.84	-5.66
BV	3.47	5.06	+45.82	3.26	-6.05	2.8	-19.31

TEAA=Total Essential Amino Acid, TNEAA=Total Non-Essential Amino Acid, EAAI=Essential Amino Acid Index, BV=Biological Value, ND= Not Detected, TAA= Total Amino Acids

Table 6. Amino acids composition of irradiated and non-irradiated Artichoke leafs

Amino acid	Control	10 KGy		20 KGy		30 KGy	
	g/100g	g/100g	% Change	g/100g	%change	g/100g	% Change
Aspartic	1.82	1.68	-7.69	1.61	-11.54	2.17	+19.23
Threonine	0.91	0.77	-15.38	0.77	-15.38	0.98	+7.69
serine	0.77	0.63	-18.18	0.7	-9.09	0.91	+18.18
Glutamic	0.91	0.77	-15.38	0.84	-7.69	0.98	+7.69
Proline	0.84	0.77	-8.33	0.7	-16.67	0.7	-16.67
Glycine	0.56	0.42	-25.00	0.56	0.00	0.56	0
Alanine	0.49	0.42	-14.29	0.49	0.00	0.56	+14.29
Cystine	0.28	0.21	-25.00	0.21	-25.00	0.21	-25.00
Valine	0.77	0.7	-9.09	0.91	+18.18	0.91	+18.18
Methionine	ND	ND	-	0.07	+0.07	ND	-
Isoleucine	0.77	0.7	-9.09	0.98	+27.27	0.91	+18.18
Leucine	2.73	2.45	-10.26	2.94	+7.69	3.15	+15.38
Tyrosine	0.56	0.42	-25.00	0.56	0.00	0.56	0.00
Phenylalanine	0.98	0.91	-7.14	0.98	0.00	1.05	+7.14
Histidine	0.91	0.84	-7.69	0.84	-7.69	0.91	0.00
Lysine	1.33	1.19	-10.53	1.26	-5.26	1.33	0.00
Arginine	0.98	0.91	-7.14	0.91	-7.14	1.05	+7.14
TAA	15.61	13.86	-11.21	15.4	-1.35	17.01	+8.97
TEAA	9.38	8.47	-9.70	9.59	+2.24	10.29	+9.70
TNEAA	6.23	5.32	-14.61	5.67	-8.99	6.65	+6.74
EAAI	15.41	13.64	-11.49	15.1	-2.01	16.11	+4.54
BV	9.15	7.14	-21.97	8.8	-3.83	9.95	+8.74

TEAA=Total Essential Amino Acid, TNEAA=Total Non-Essential Amino Acid, EAAI=Essential Amino Acid Index, BV=Biological Value, ND= Not Detected, TAA= Total Amino Acids

Irradiation processing at all dose levels caused a significant effects on fatty acid profile of the two agro industrial wastes, these effects appears in the increase or decrease of fatty acids with no

fixed pattern, and some fatty acids not detected in non-irradiated samples of artichoke leaves and appeared in irradiated samples as myristic, stearic and arachidic and others were disappeared as myristic fatty acid in samples irradiated at dose level 10 kGy and oleic fatty acid in samples irradiated at dose level 30 kGy.

Inspite, irradiation treatment caused increase and decrease in fatty acids but the overall effect in most irradiated samples was the increase in essential fatty acids except for grape seed-skin and artichoke leaves irradiated by 30 kGy of γ-rays. The fluctuating values of fatty acids in irradiated samples agree with Silva, et al. [86], in their study on *Echinodorus macrophyllus* exposed to γ-radiation and also irradiation cause the formation of free radicals which react with poly unsaturated fatty acids producing unstable hydroperoxides and a range of further degradation products.

The irradiation treatment causes a decrease of fatty acid and an increase of trans fatty acid, which occur due to a change in the molecular structure of fatty acid, breaking down double bonds, forming free radical and trans fatty acids (Afify et al. [87]).

3.3 Effect of Feeding Male Wistar Rats on Experimental Diets on the Body Weight Gain

Results in Fig. 1 showed that rats fed on reference diet for 58 days (about 8 weeks) had the lowest final weight, total weight gain and daily weight gain. On the other hand, rats fed on reference diet with 20% fat and diets supplemented with non-irradiated and irradiated agro-industrial byproducts under investigation, has higher values (>3 folds for grape seeds-skin and >2 folds for artichoke leaves) for the same parameters.

The lowest values of final weight, total weight gain and daily weight gain parameters between rats fed on diets supplemented with non-irradiated and irradiated agro-industrial byproducts, was obtained from rats fed on non-irradiated and irradiated artichoke leaves. These were attributed to the highest content of tannins (0.09 mg/g), and the highest fiber content (461.7-534.3 g/kg) among agro-industrial wastes under investigation.

Feeding rats on diets supplemented with non-irradiated and irradiated grape seeds-skin showed values of final weight, total weight gain and daily weight gain intermediate between two other byproducts. These results may be because grape seeds-skin contains lower tannins (0.03-0.05 mg/g) and lowest fiber content (153.7-169.3 g/kg). Results obtained in these parts agree with results obtained by El- Neily and El-Shenawy [88].

3.4 Effect of Feeding Male Wistar Rats Experimental Diets on Certain Serum Biochemical Parameters

Rats fed on reference diet with 20% fat had the highest values of tested parameters as serum cholesterol, triglycerides, low density lipoprotein (LDL-C), very low density lipoprotein (VLDL-C), atherogenic index (AI), glucose, liver function parameters AST, ALT and AST/ALT ratio, while high density lipoprotein (HDL-C) showed lowest values, leading to higher risk for atherosclerosis and coronary heart disease. On the other hand, supplementing high fat diets with non-irradiated or irradiated two agro industrial wastes, had positive effects on rats' health with different ratios (Tables 8,9).

Table 7. Fatty acids percentage of raw and gamma irradiated wastes

Agro industrial wastes	Fatty acid	Radiation dose (kGy)						
		0	10		20		30	
		%	%	% Change	%	% Change	%	% Change
Grape seeds-skin	Palmetic	33.08	31.63	-4.38	11.45	-65.39	28.67	-13.33
	Stearic	10.52	10.98	+4.37	4.75	-54.85	14.48	+37.64
	Oleic	41.23	36.65	-11.11	26.16	-36.55	18.86	-54.26
	Linoleic	15.18	20.74	+36.63	56.43	+271.74	ND	--
Artichoke leaves	Myristic	6.44	ND	--	6.47	+0.47	3.95	-38.66
	Palmetic	67.61	84.64	+25.19	67.77	+0.24	52.78	-21.93
	Stearic	ND	ND	--	ND	--	8.85	+100
	Arachidic	ND	ND	--	ND	--	3.14	+100
	Oleic	14.07	15.36	+9.17	13.44	-4.48	ND	--

ND= Not detected

Table 8. Some biochemical parameters of rats fed on non-irradiated and irradiated Grape seed-skin incorporated in high fat diet

Parameters	Experimental diets							
	D1	D2	D3	D4	D5	D6	Pooled SE	P value
Total Cholesterol, (mg/dl)	145.58^{b}±1.52	166.54^{a}±1.802	144.9^{b}±0.727	138.9^{c}±0.605	135.73^{c}±1.253	144.56^{b}±0.048	1.221	0.0001
Triglycerides, (mg/dl)	85.03^{c}±0.815	120.87^{a}±4.666	97.6^{b}±0.085	94.25^{b}±0.836	93.2^{b}±0.033	83.49^{c}±0.955	1.421	0.0001
HDL-C, (mg/dl)	99.7^{b}±0.268	95.58^{c}±0.054	99.4^{b}±0.955	101.33^{b}±0.433	105.1^{a}±2.236	106.18^{a}±0.487	1.034	0.0001
LDL –C, (mg/dl)	26.2^{bc}±0.406	47.14^{a}±3.729	27.06^{b}±1.443	18.95^{d}±1.166	19.74^{d}±0.052	20.89^{cd}±0.609	1.776	0.0001
VLDL-C, (mg/dl)	17.0^{d}±0.162	24.2^{a}±0.939	19.5^{b}±0.017	18.83^{bc}±0.164	18.62^{c}±0.015	16.7^{d}±0.202	0.287	0.0001
A.I.	0.43^{b}±0.004	0.75^{a}±0.022	0.45^{b}±0.011	0.37^{c}±0.01	0.29^{d}±0.016	0.36^{c}±0.008	0.019	0.0001
Glucose, (mg/dl)	90.01^{d}±2.178	122.73^{a}±0.20	113.07^{b}±0.2	110.95^{bc}±0.337	108.86^{c}±1.364	108.11^{c}±0.1	1.099	0.0001
AST, (U/L)	37.92^{c}±0.115	42.63^{a}±0.036	41.24^{b}±0.046	40.93^{b}±0.136	41.08^{b}±0.119	41.02^{b}±0.06	0.09	0.0001
ALT, (U/L)	38.18^{e}±0.13	44.68^{a}±0.125	42.72^{b}±0.126	40.68^{c}±0.169	40.46^{c}±0.277	39.85^{d}±0.24	0.189	0.0001
AST, ALT ratio	0.99^{b}±0.003	0.95^{c}±0.004	0.95^{c}±0.009	1.00^{ab}±0.004	1.02^{a}±0.006	1.02^{a}±0.007	0.005	0.0001

D1= Reference diet, D2= Reference diet plus 20% fat, D3= High fat diet incorporated with raw (non- irradiated) grape seed-skin, D4= High fat diet incorporated with 10 kGy irradiated grape seed-skin, D5= High fat diet incorporated with 20 kGy irradiated grape seed-skin, D6= High fat diet incorporated with 30 kGy irradiated grape seed-skin.
Values are means ± SE. Means in the same Raw with the same letter are not significantly different (P<0.05).

Table 9. Some biochemical parameters of rats fed on non-irradiated and irradiated Artichoke leaves incorporated in high fat diets

Parameters	Experimental diets							
	D1	D2	D3	D4	D5	D6	Pooled SE	P value
Total Cholesterol, (mg/dl)	145.58^{b}±1.52	166.54^{a}±1.802	145.7^{b}±1.310	135.39^{c}±1.100	137.98^{c}±3.453	137.7^{c}±4.847	2.100	0.0001
Triglycerides, (mg/dl)	85.03^{c}±0.815	120.87^{a}±4.666	96.78^{b}±0.205	90.64^{c}±2.900	90.33^{c}±1.856	88.52^{c}±0.693	1.982	0.0001
HDL-C, (mg/dl)	99.7^{b}±0.268	95.58^{c}±0.054	101.84^{b}±0.441	101.9^{b}±0.300	117.67^{a}±1.444	115.83^{a}±1.799	0.952	0.0001
LDL–C, (mg/dl)	26.2^{bc}±0.406	47.14^{a}±3.729	24.49^{b}±1.566	15.34^{c}±0.800	13.37^{c}±0.770	17.0^{c}±0.306	1.496	0.0001
VLDL-C, (mg/dl)	17.0^{d}±0.162	24.2^{a}±0.939	19.34^{b}±0.04	18.12^{c}±0.6	18.07^{c}±0.4	17.7^{c}±0.14	0.396	0.0001
Atherogenic Index	0.43^{b}±0.004	0.75^{a}±0.022	0.43^{b}±0.018	0.33^{c}±0.010	0.17^{d}±0.015	0.17^{d}±0.015	0.014	0.0001
Glucose, *(mg/dl)*	90.01^{c}±2.178	122.73^{a}±0.20	108.17^{b}±1.644	111.78^{b}±0.030	109.42^{b}±0.282	110.87^{b}±0.361	1.416	0.0001
AST, (U/L)	37.92^{c}±0.115	42.63^{a}±0.036	40.61^{b}±0.188	40.41^{b}±0.164	40.91^{b}±0.470	40.31^{b}±0.360	0.197	0.0001
ALT, (U/L)	38.18^{d}±0.13	44.68^{a}±0.125	41.79^{b}±0.250	40.89^{c}±0.187	40.73^{c}±0.240	40.55^{c}±0.210	0.173	0.0001
AST, ALT ratio	0.99^{b}±0.003	0.95^{c}±0.004	0.97^{cd}±0.002	0.98^{bc}±0.006	1.01^{a}±0.005	1.0^{ab}±0.011	0.005	0.0001

D1= Reference diet, D2= Reference diet plus 20% fat, D3= High fat diet incorporated with raw (non- irradiated) artichoke leaves, D4= High fat diet incorporated with 10 kGy irradiated artichoke leaves, D5= High fat diet incorporated with 20 kGy irradiated artichoke leaves, D6= High fat diet incorporated with 30 kGy irradiated artichoke leaves.
Values are means ± SE, Means in the same Raw with the same letter are not significantly different (P<0.05)

Best lipid profile results were obtained from rats fed on diet supplemented with irradiated artichoke leaves, taking in consideration that there was no significant difference between samples irradiated by different doses. Serum HDL-C of rats fed on diet supplemented with artichoke leaves irradiated by 20kGy of γ- rays has the highest value leading to the lowest risk factor of atherosclerosis among rats fed on experimental diets.

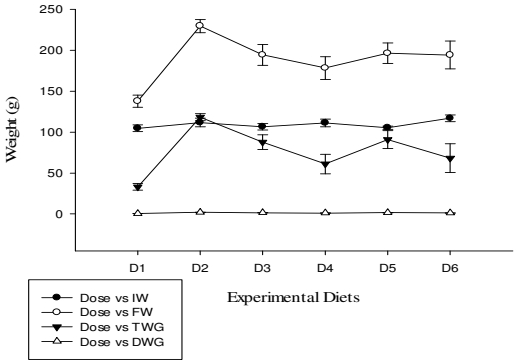

Fig. 1. Body mass of rats fed on non-irradiated and irradiated Grape seed-skin incorporated in high fat diets

D1= Reference diet, D2= Reference diet plus 20% fat, D3= High fat diet incorporated with raw (non-irradiated) grape seed-skin, D4= High fat diet incorporated with 10 kGy irradiated grape seed-skin, D5= High fat diet incorporated with 20 kGy irradiated grape seed-skin, D6= High fat diet incorporated with 30 kGy irradiated grape seed-skin.
IW= Initial weight, FW= Final weight, TWG= Total weight gain, DWG= Daily weight gain.
Values are means ± SE.

Results of glucose also revealed that feeding rats on non-irradiated and irradiated artichoke leaves has lowering effect giving values between those of rats fed reference diet with 20% fats and rats fed on reference diet.

Supplementing diets with non-irradiated and irradiated grape seed-skin has also positive effects on health of rats fed high fat diets. The lowering effect of liver function parameters were almost the same.

Overall results of the *in vivo* studies can be explained by that artichoke leaves contains the highest polyphenols, tannins and the highest fiber contents so possess the highest free radical scavenging activity. In case of rats fed on grape seed-skin the presence of poly phenols and tannins were predominant than fiber content.

The anti-atherogenic effect of grape seeds was due to the presence of the total phenolic compounds and polyunsaturated fatty acids [89, 90]. The major total fatty acids present in grape seeds oils are unsaturated fatty acids, which play a crucial role in reducing blood cholesterol in human and rats [91].

Results are in good agreement with Jiao et al. [92], who observed the hypocholesterolemic activity of grape seeds and explained this effect by the enhancement of bile acid excretion mediated by grape seeds, results obtained in this study agrees with, Bundy et al. [93]; Gebhardt [94,95]. The authors reported that artichoke leaves extract helps in reduction of plasma total cholesterol in adults with mild to moderate hypercholesterolemia, due to the presence of luteolin, one of artichoke leaves extract constituents which inhibits the *de novo* synthesis of cholesterol and increases biliary secretion from the liver. The same explanation of lipids lowering effect of artichoke leaves extracts obtained by Gebhardt and Fausel, [96] because the leaves inhibit hepatocellular cholesterol biosynthesis so they indirectly help to reduce the atherogenic risk.

4. CONCLUSION

The growing male Wistar rats were tested for physiological and biochemical responses; they were kept for 8 weeks on diets supplemented with non-irradiated or irradiated agro-industrial wastes. The results showed that there is no critical changes were observed due to irradiation. Further researches is needed to be conducted to estimate the individual effect of radiation processing on each bioactive compound present in the extract of grape seed-skin and artichoke leaves. This research article may be of real benefit for recycling agro-industrial wastes to be used as diets supplements for animals. Furthermore, it may minimize the harmful effects of junk food on human as well, however, further studies should be done.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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