

Original Research Article

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

Abstract:

Food irradiation is a method of preservation; it is 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. The present study was conducted to test the possible biochemical impacts of radiation processing on wastes of grapes and artichoke, as well as to elucidate the physiological and biochemical effects of feeding growing male Wister rats on diets supplemented with the processed agro industrial wastes at dose levels of 10, 20 and 30 kGy. The chemical composition of processed agro industrial wastes at the above mentioned doses showed no significant differences in corresponding to the non-irradiated one. Irradiation processing caused an increase in polyphenols as a function of radiation dose, meanwhile grape seed-skin processed by 20 kGy gamma rays showed a decrease by 15.38%. Tannin content exhibited an increase in both grape seed-skin and artichoke leaves at all radiation doses used, except in grape seed-skin irradiated at dose level 10 kGy has lost 25 % corresponding to their control one. Regarding to the impact of irradiation treatment up to 30 kGy on free radical scavenging activity of grape seeds-skin, and artichoke leaves, the results indicated 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. It was observed that the changes in amino acids and fatty acids patterns as affected by irradiation up to and including 30 kGy was occurred without a specific trend in the two agro industrial wastes under investigation. The physiological and biochemical performance of growing male Wister rats as affected by feeding high fat diets supplemented with non-irradiated or irradiated up to and including 30 kGy agro industrial wastes under investigation for 8 weeks, showed better results when compared with those rats fed on reference diet. It has been manifested in body weight gain and internal organ weight alongside biochemical aspects such as serum total cholesterol, triglycerides, LDL, HDL, VLDL, AI, ALT, AST and plasma glucose.

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 ($OH^\cdot$), 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 can lead to chronic inflammation, which in turn could mediate most chronic diseases including cancer, diabetes, and 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⁾. Therefore, Antioxidants are believed to play a very important role in the body defense system against ROS 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-butylhydroquinone (TBHQ), are widely used in the food industry as potential antioxidants ⁵⁾. However, natural antioxidants might help to attenuate the oxidative processes ⁶⁾, mainly from plant origin which 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⁾. Polyphenols are probably the most investigated molecules of nutritional interest; polyphenols are natural antioxidants with an interesting future in various fields such as food and medicine ¹⁾. It is becoming clear that the regular consumption of these antioxidant rich foods in our daily life can be able to prevent free radical mediated degenerative diseases ²⁾.

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 species*) are members of the family *Vitaceae*. They are among the most important plant species cultivated, with an area of about 7.4 million hectare worldwide. 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

85 production of added value products^(25, 26, 27, 28, 29). The utilization of by-products
86 from wine making is an urged problem in Europe within context of recently
87 posed regulations presuming measures against burying of wine by-products
88 affecting the soil erosion/compaction and the quality of groundwater³⁰⁾.

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

94 Globe artichoke has a low content of fat and high levels of minerals
95 (potassium, sodium, and phosphorus), vitamin C, fibers, polyphenols, flavones,
96 inulin and hydroxycinnamates - caffeoylquinic acid derivatives^{36, 37, 38)}.
97 According to **Foti et al.**³¹⁾ the chemical composition of artichoke seeds was the
98 following: crude protein 21.6%, crude fiber 17.1%, crude oil 24.05% and ash
99 3.8%. The artichoke flowerheads have a high content of vitamin C (10 mg / 100
100 g FW) and minerals (K 360 mg / 100 g FW; Ca 50 mg / 100g FW)³⁶⁾, where
101 FW is fresh weight. Leaves and heads of artichoke have been found to be rich in
102 polyphenolic compounds, inulin, fiber and minerals³⁵⁾.

103 However, Plant materials are highly susceptible to microbial contamination
104 due to the medium (water and soil) in which they grow, harvesting and storage.
105 Processing may cause additional contamination and microbial growth³⁹⁾. Thus,
106 an adequate technology for decontamination is required to improve the hygienic
107 quality of plant materials and to make it suitable for human use and
108 commercialization^{39, 40)}.

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

118 Ionizing radiations (γ and e-beams) and UV light can inactivate food borne
119 microorganisms without substantially heating the food⁴³⁾. The use of γ -
120 irradiation to enhance the shelf life of minimally processed fruits and vegetables
121 and to ensure the microbiological safety is increasing because it appears to be
122 effective both on cells and on spores. On the other hand, food irradiation can be
123 used to reduce insect infestation of grain, dried spices, and dried or fresh fruits
124 and vegetables; inhibit sprouting in tubers and bulbs; retard postharvest ripening
125 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⁾.

Materials and methods:

Materials:

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

Preparation of Samples:

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

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 level 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 4°C until used.

Animals:

A total number of 60 Wister 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 veterinary. The animals were healthy and clinically free of internal and external parasites. Animals were kept in the animal house at an ambient temperature 22 °C ±3 and 50±5 % relative humidity with a 12 h 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.

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.

Methods:-

Chemical composition:

Moisture content, crude fat, crude protein, ash content was 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:

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

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 150°C for 1 min then elevated to 235 °C with a rate of 17 °C/ min then, raised again to 245 °C with a rate of 1°C /min and hold at 245 °C for 5 min. gasses flow rates for N₂ as a carrier gas, H₂ and air were 1.3, 40 and 400 ml /min, respectively. Flame ionization detector and injection temperatures were 275 °C and 260 °C, respectively.

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 110°C 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 filter⁵⁰⁾. 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 chromeTM chromatography data system tutorial and user guide-version 6.7.

Tannins analysis: It was determined according to, **Balbaa**⁵¹⁾

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⁾.

Total Phenolic compounds: Extraction and determination of Phenolic compounds was conducted according to the method described by **Daniel and George**⁵³⁾.

Biochemical assays:

Serum ALT and AST were determined according to **Sherwin and Pesce**⁵⁴⁾. Determination of total serum cholesterol was done according to **Richmond**⁵⁵⁾, Serum HDL- Cholesterol⁵⁶⁾, 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/5⁵⁸⁾, serum very low density lipoprotein cholesterol (VLDL-C) was calculated by the equation: Serum VLDL-C mg/dl = Triglycerides/5⁵⁹⁾. Atherogenic Index (AI) was calculated using the equation, AI = (total cholesterol-HDL-C)/HDL-C⁶⁰⁾, Plasma glucose was measured by the method of **Caraway and Watts**,⁶¹⁾.

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 was separated using the Duncan's Multiple Range Test⁶²⁾. The α -level for significance was $P \leq 0.05$.

Results and Discussion:

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 easily to be radiolyzed by irradiation to produce enough free radicals that could induce significant changes in 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 state⁶³⁾. Crude fiber of artichoke leaves was significantly decreased as radiation dose increase from 10 kGy to 30 kGy. On the other hand, nitrogen free extract (NFE) content increases as radiation dose increases; 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, and in good agreement with **El-Niely**⁶⁶⁾; **Ali**⁶⁷⁾ and **Farag**⁶⁸⁾, the authors 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 kGy⁶⁹⁾, irradiation processing of cotton seed at 20, 40, 60 and 80 kGy⁷⁰⁾, canola meal or cassava meal irradiated at dose levels of 50, 75 and 100 kGy⁷¹⁾, Artichoke by-product irradiation at 75 and 100 kGy⁷²⁾ 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 obvious 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 polymers⁷³⁾, so 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 polyphenols⁷⁴⁾.

Results in the present study of polyphenols agree with **Harrison and Were**,⁷³⁾ who mentioned that γ -irradiation increased the total phenolics yield in almond skin extracts 45% at dose levels of 4 kGy and above when compared to the control (non-irradiated), and attributed their results to the release of phenolic compounds from glycosidic components and the degradation of larger phenolic compounds into smaller ones by γ - irradiation.

Results of grape seeds-skin irradiated at dose level 10 kGy, agrees with previous studies using γ - irradiation,^{75,76)} on different types of legumes (peas, cowpeas, lentils, kidney beans, and chickpeas), caused decrease in tannins, while results of grape seeds-skin irradiated at dose levels 20 and 30 kGy and artichoke at all dose levels used agrees with **Stajner et al.**,⁷⁷⁾ observed increase of tannin content of soybean seeds by γ - irradiation at dose between 1 to 10 kGy

and **Harrison and Were**,⁷³⁾ observed the same effect on almond skin extracts at doses below 4 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 spices⁷³⁾.

In most studies using ionizing irradiation treatment of food stuffs, a decrease in the level of 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 was increased (**Table 4**). The decrease in 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)}.

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 amino acids alanine and cystine were 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 both were decreased. 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 seed-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 and 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 acids⁸²⁾.

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 bonds⁸²⁾. Where, simple amino acids due to irradiation undergo reductive deamination and decarboxylation, aliphatic amino acids with increasing chain length, providing additional C-H bond for interaction with OH[•] radical reduces the amounts of oxidative deamination⁸³⁾, 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 reaction⁸⁴⁾. **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.

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.

These results are in good agreement with Afify *et al.*,⁸⁷⁾, who stated that 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.

Effect of feeding male Wister rats on experimental diets on the body weight gain:

Results in **Figure 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 (461.7-534.3g/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 (153.7-169.3g/kg). Results obtained in these parts agree with results obtained by El- Neily and El-Shenawy⁸⁸⁾.

Effect of feeding male Wister 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), very low density lipoprotein (VLDL), atherogenic index (AI), glucose, liver function parameters (AST, ALT) and AST/ALT ratio, while high density lipoprotein (HDL) 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**).

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 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.

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 polyphenol and tannin concentrations so possess the highest free radical scavenging activity and highest fiber content, followed by grape seed-skin, but in case of rats fed on grape seed-skin the presence of poly phenols and tannins were predominant factor 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 rats⁹¹⁾.

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, duo 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.

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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
<u>Calculated analysis</u>	
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 D₃, 40 IU of vitamin E, 4.5 mg of vitamin B₁, 9 mg of vitamin B₂, 9 mg of Vitamin B₆, 0.04 mg of vitamin B₁₂, 85 mg of niacin, 27 mg of D-pantothenic acid, 0.4 mg of biotin, and 1.2 mg of folic acid.

694 **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		
Proteins¹	121.9 ^a ±1.2	117.6 ^a ±0.66	118.2 ^a ±1.50	121.1 ^a ±0.75	0.1117	0.1232
Fiber²	153.7 ^b ±3.38	169.3 ^a ±2.96	168.3 ^a ±0.33	158.0 ^b ±2.73	2.6352	0.0073
Ash³	29.9 ^a ±1.27	31.2 ^a ±0.93	28.7 ^a ±1.64	29.6 ^a ±0.67	1.1836	0.5118
Fat⁴	124.0 ^a ±4.00	132.3 ^a ±1.45	124.3 ^a ±4.25	133.2 ^a ±2.31	0.2502	0.1040
Moisture⁵	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 Extract⁶	462.3 ^a ±12.37	441.7 ^a ±3.73	451.6 ^a ±6.11	450.9 ^a ±2.57	7.2653	0.3268

695 Values are means ± SE.

696 Means in the same Row with the same letter are not significantly different.

697

698 **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		
Proteins¹	208.3 ^a ±1.3	198.5 ^a ±1.3	200.7 ^a ±5.5	204.5 ^a ±4.6	3.7127	0.3216
Fiber²	534.3 ^a ±2.9	517.7 ^b ±1.5	516.0 ^b ±3.1	461.7 ^c ±6.0	3.7527	0.0001
Ash³	60.0 ^a ±1.2	61.4 ^a ±2.3	59.1 ^a ±2.0	65.4 ^a ±1.8	1.8644	0.1624
Fat⁴	11.6 ^a ±0.1	11.2 ^b ±0.0	10.5 ^c ±0.2	10.5 ^c ±0.0	0.1213	0.0005
Moisture⁵	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 Extract⁶	159.7 ^c ±2.3	181.6 ^b ±3.2	186.1 ^b ±1.2	227.9 ^a ±9.4	5.1490	0.0001

699 Values are means ± SE.

700 Means in the same Row with the same letter are not significantly different.

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707 **Table (4): Antioxidant content and activity of raw and gamma irradiated grape seeds-skin and**
708 **artichoke leaves.**

	Antioxidant Parameter	Dose (kGy)				Pooled SE	P-Value
		0	10	20	30		
Grape seeds-skin	Total Phenolic Compounds¹ (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)		
	Tannins² (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)		
Artichoke leaves	DPPH³ (%)	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)		
	Total Phenolic Compounds¹ (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
Artichoke leaves	Change (%)		(+12.00)	(+4.00)	(+10.67)		
	Tannins² (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)		
Artichoke leaves	DPPH³ (%)	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)		

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710 *Values are means ± SE.*

711 *Means in the same Row with the same letter are not significantly different.*

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Table (5): Amino acids composition of irradiated and non-irradiated grape seed - skin

Amino acid	Control g/100g	10 KGy		20 KGy		30 KGy	
		g/100g	% Change	g/100g	% Change	g/100g	% 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 leaves

<i>mino acid</i>	<i>Control g/100g</i>	10 KGy		20 KGy		30 KGy	
		<i>g/100g</i>	<i>% Change</i>	<i>g/100g</i>	<i>% Change</i>	<i>g/100g</i>	<i>% Change</i>
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

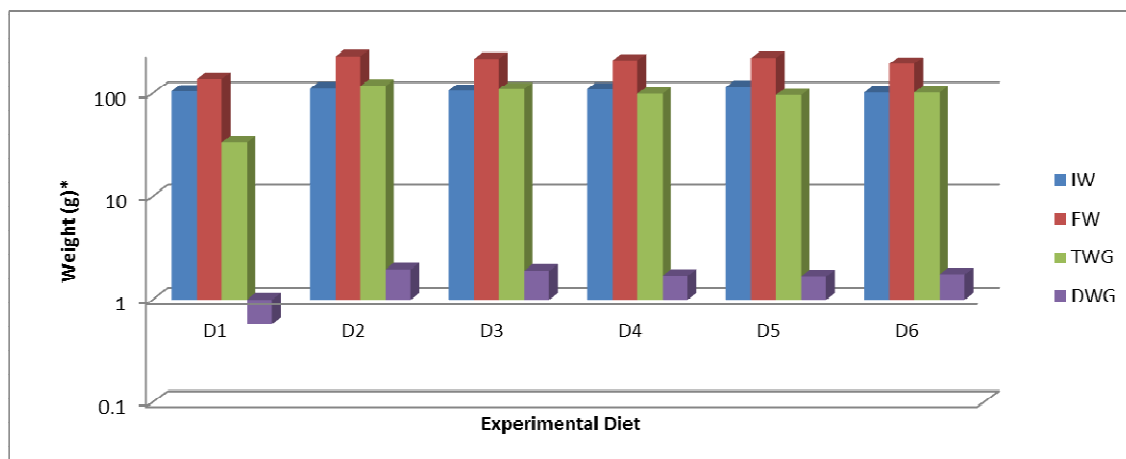
TEAA=Total Essential Amino Acid, TNEAA=Total Non-Essential Amino Acid, EAAI=Essential Amino Acid Index, BV=Biological Value, ND= Not Detected, TAA= Total Amino Acids

Table (7): Fatty acids percentage of raw and gamma irradiated wastes

Agro industrial wastes	Fatty acid	Radiation Dose (kGy)						
		0	10		20		30	
		<i>%</i>	<i>%</i>	<i>% Change</i>	<i>%</i>	<i>% Change</i>	<i>%</i>	<i>% Change</i>
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	--

720 *ND= Not detected.*



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Figure (1): Body mass of rats fed on non-irradiated and irradiated Grape seed-skin incorporated in high fat diets

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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 $\pm$ SE.

Means in the same Row with the same letter are not significantly different ($P < 0.05$).

735 **Table (8): Some biochemical parameters of rats fed on non-irradiated and irradiated Grape**
736 **seed-skin incorporated in high fat diet**

<i>Parameters</i>	Experimental Diets							P Value
	D1	D2	D3	D4	D5	D6	Pooled SE	
<i>Total Cholesterol, (mg/dl)</i>	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
<i>Triglycerides, (mg/dl)</i>	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
<i>HDL-C, (mg/dl)</i>	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
<i>LDL -C, (mg/dl)</i>	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
<i>VLDL-C, (mg/dl)</i>	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
<i>A.I.</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
<i>Glucose, (mg/dl)</i>	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
<i>AST, (U/L)</i>	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
<i>ALT, (U/L)</i>	38.18 ^c ±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
<i>AST, ALT ratio</i>	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

737 *D1= Reference diet, D2= Reference diet plus 20% fat, D3= High fat diet incorporated with raw (non-*
738 *irradiated) grape seed-skin, D4= High fat diet incorporated with 10 kGy irradiated grape seed-skin, D5=*
739 *High fat diet incorporated with 20 kGy irradiated grape seed-skin, D6= High fat diet incorporated with 30*
740 *kGy irradiated grape seed-skin.*

741 *Values are means ± SE.*

742 *Means in the same Row with the same letter are not significantly different (P<0.05).*

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763 **Table (9): Some biochemical parameters of rats fed on non-irradiated and irradiated Artichoke**
764 **leaves incorporated in high fat diets**

Parameters	Experimental Diets							P Value
	D1	D2	D3	D4	D5	D6	Pooled SE	
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
LDL-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 ^d ±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 ^e ±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

765 D1= Reference diet, D2= Reference diet plus 20% fat, D3= High fat diet incorporated with raw (non-
766 irradiated) artichoke leaves, D4= High fat diet incorporated with 10 kGy irradiated artichoke leaves, D5=
767 High fat diet incorporated with 20 kGy irradiated artichoke leaves, D6= High fat diet incorporated with 30
768 kGy irradiated artichoke leaves.

769 *Values are means ± SE.*

770 *Means in the same Row with the same letter are not significantly different (P<0.05).*