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Effect of Gamma Irradiation Doses on Some Chemical Characteristics of Cotton Seed Oil

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COTTON SEEDS c.v. Giza 85 (*Gossypium hirsutum* L.) were exposed to gamma irradiation doses of 0.5, 1.0 and 1.5 kGy to improve some chemical characteristics of cotton seed oil i.e. saturated and unsaturated fatty acids, gossypol and *β*-sitosterol that were bound oil. The presented study showed that, the saturated fatty acids; lauric, palmitic and stearic increased when the cotton seeds were exposed to gamma irradiation doses of 0.5 up to 1.5 kGy, On the other hand, arachidic acid content decreased in all the irradiated treatments compared with untreated cotton seed. The unsaturated fatty acid oleic was increased in irradiated cotton seed samples compared with untreated one, while linoleic, the major unsaturated fatty acid decreased in irradiated cotton seed oil than untreated seeds. Gossypol and *β*-sitosterol, bound oil, in irradiated cotton seeds increased gradually with gamma irradiated doses compared with untreated control samples.

Keywords: *Cotton seed, oil, fatty acids, gossypol, β-sitosterol*

Gamma rays belong to ionizing radiation and interact with atoms or molecules to produce free radicals in cells. These radicals have been reported to affect the morphology, anatomy, biochemistry and physiology of plants and can cause damage or modify the plant cell components depending on the irradiation level. These effects include various changes e.g., dilation of thylakoid membranes, alteration in photosynthesis and modulation of antioxidative system (Wi *et al.*, 2005).

Oil of cotton seed has long been considered to be a good vegetable oil for frying but their use was limited as the oxidative stability of cotton seed oil can be lower than other vegetable oils. This is because of its high content of linoleic acid (18:2). To improve the oil stability, cotton seed can be partially hydrogenated which reduces the level of linoleic acid C18:2 (Sacks and Katan, 2002). In addition, the presence of high level content of saturated fatty acid, palmitic (16:0), in cotton seed oil (~27%) contribute functionality in food systems, and they also contribute negatively to serum cholesterol profiles (Zock *et al.*, 1994). Also, cotton seed oil retained the highest frying period and long storing time because of its high content of natural antioxidants, namely tocopherols and vitamin E (Srinivasan, 2004). Antioxidants present in cotton seed oil work against the free radicals that cause cell damage aging (Sekhar and Bhaskara, 2011). Cotton and related species contain gossypol, a polyphenolic compound, which act as an integral part of the cotton plant's self-defence system against insect pests and possibly some diseases (Jodi and Gabriela, 2008). Free gossypol is an anti-nutritional factor that limits the use of cotton seed and its products (Hron *et al*., 1987). Also it is harmful as it blocks spermatogenesis and reduces sperm motility (Sharma *et al*., 1999). The toxic effect of gossypol can be used against the cancerous cells. It has been found to have antiproliferative activity on tumour cells and is thought to be a potential anticancer drug (Wang *et al*., 2000), antimalarial drug and potential drug for human immunodeficiency virus (HIV) infection (Luna *et al.,* 2004). Part of gossypol tends to react with many natural substances in cotton seed and forms the bound gossypol which is non-harmful. *β*-sitosterol, a known plant sterol, has been reported to be abundant in oils of wheat germ, cotton seed, corn, and soybean (Chen, 1991). The structures of *β*-sitosterol and cholesterol are quite similar, so it is reasonable that *β*-sitosterol can inhibit the absorbance of cholesterol in the plasma (Mac-Latchy *et al.*, 1995) and in the body (Tatu *et al.*, 2002). *β*-sitosterol is a factor used to form the lympho cells and natural killer cells in the immunity process circulation (Bouic *et al.*, 1996). Also, it reduces prostate cancer and colon-cancer cell growth (Awad and Fink, 2000). In addition, *β*-sitosterol can improve liver function activity (Zak *et al.*, 2005).

The aim of the present work is to study the effect of γ-irradiation on cotton seeds with respect to its fatty acids; gossypol and *β*-sitosterol bound oil contents.

Material and Methods

Cotton seeds

Gossypium hirsutum L. cv. Giza 85 obtained from Beni sweif governorate exposed to γ-rays doses of 0.5, 1, and 1.5 kGy. Irradiation was done by a ^{60}Co Hendy Cell Research, NCRRT, Cairo, Egypt at a dose rate of 3.61 kGy/h.

Lipid extraction

Lipid extraction from samples was conducted according to the method of A.O.A.C. (2000), using chloroform methanol (2:1 v/v). The associated non-lipids were removed by washing lipid extract three times with CH_3 OH: H_2O (1:1 v/v). The lipids, chloroform layer, were dried over anhydrous sodium sulfate, and the solvent was evaporated by heating at 40 °C under vacuum.

Separation of fatty acids and β-sitosterol.

Lipid samples were saponified, overnight, with alcoholic KOH (20%) at room temperature:

i. **Separation of β-sitosterol:** The unsaponified fraction, containing β-sitosterol, was extracted with 4x10mL petroleum ether and all the extracts were later combined. The extract was washed with 0.5N NaOH and repeatedly with distilled water until neutral. Traces of moisture were removed by the addition of sufficient amount of anhydrous sodium sulphate. Finally, the extract was evaporated to remove the petroleum ether and redissolved in absolute ethanol.

ii. **Separation of fatty acids:** The fatty acids were freed from the saponified fraction by acidification with hydrochloric acid (N), followed by extraction with ether. The ether extract was washed three times with distilled water, then dried over anhydrous sodium sulphate, and filtered off (Vogel, 1975).

Preparation of diazomethane

Diazomethane was prepared from methylamine hydrochloride, Vogel (1975).

Methylation of fatty acids with diazomethane

Fatty acids liberated from lipid samples and standard fatty acids samples were dissolved in 1 ml anhydrous methanol and the ethereal solution of diazomethane was added in a small portion until the mixture acquired a pale yellow colour and gas evolution ceased. The reaction mixture was left for 10 min and ether was evaporated under nitrogen stream at room temperature. 2 drops of redistilled chloroform solution was added to dissolve the fatty acid methyl esters and 1µl of this solution was injected into the gas chromatograph instrument.

Sources of standard fatty acids

Standard fatty acids (12:0, 14:0, 16:0, 18:0, 18:1, 18:2 and 20:0) with a stated purity of 99% by Gas Liquid Chromatography (GLC) was purchased from Nu-check Prop. The purity of each fatty acid methyl ester was checked by GLC and gave one peak.

Identification and determination of fatty acids by gas liquid chromatography (GLC)

Fatty acids content of the irradiated cotton seeds were determined at Faculty of Agriculture Research Park (FARP), Faculty of Agriculture, Cairo University according to Farag *et al.* (1981). Samples were analyzed by GLC (Pye Unicam Series 304), equipped with a flame detector, as follows: glass column (1.5m x 4mm) packed with Diatomite (100-120 mesh) and coated with 10% polyethylene glycol adipate (PEGA). The column oven temperature rose from 70^{O}C-190°C with 8^{O}C/min, then isothermally at 190^{O}C for 25min with nitrogenflow of 30 ml/min.

Determination of β-sitosterol and gossypol

β-sitosterol was analyzed at Faculty of Agriculture Research Park (FARP), Cairo University by Hewlett Packard, HP 1090 High Performance Liquid Chromatography (HPLC) according to Yi and Xiao (2009) using Waters 600 HPLC equipment combined with UV detector at UV 210nm. Symmetry C18 Column (5μm, 3.9×150mm). Column temperature was 30°C Mobile phase: MeOH (HPLC grade). The flow rate was 1.0ml/ min. Sample Loading was 30μl.

Gossypol Samples were prepared according to Wang *et al.* (1985). The dried powdered cotton seed samples (about 0×1g) were macerated with acetone for 16 h, then filtered through 0×45 mm micro-filter membrane and the residue washed. The extract was evaporated to dryness under vacuum. The residue was resuspended in 1% HOAc-CHCl3 solution to 25 ml, and determined according to Yingfan *et al.* (2004), through an optimized HPLC on a C18 column (4×6 mm × 250 mm, 5 mm particle) with methanol-0.5% acetic acid aqueous solution, 90: 10 (v/v), as mobile phase, at a flow rate of 0.8 ml/min and UV detection at 254 nm.

Statistical analysis

Data obtained were statistically analyzed by using CoStat statistical program software, 1990 and Duncan's multiple range test (Duncan, 1955) was applied at 5% probability level to compare the differences among time means.

Results and Discussion

Effect of gamma irradiation doses on cotton seed fatty acids

The untreated cotton seed samples contain saturated fatty acids (35%) with palmetic acid (16:0) as the major one (27%), while lauric (C 12:0), myristic (C 14:0), stearic (C 18:0) and arachidic (C 20:0) were found as a minor constituents. On the other hand, unsaturated fatty acids found to represent the main constituents (63%), where linoleic (C 18:2) and oleic (C 18:1) were the major constituents with a percentage of 40.29 and 21.98%, respectively (Table 1 & Fig. 1).

Exposure of cotton seeds to γ-irradiation with doses of 0.5, 1.0 and 1.5 kGy resulted in some modification of their fatty acid pattern. With respect to saturated fatty acids palmetic acid (16:0) content showed significant increase compared to un-irradiated cotton seeds, palmitic acid (C 16:0) percentage was increased in cotton seed lipids exposed to gamma doses of 0.5, 1.0 and 1.5 kGy with a value of 32.48, 30.61 and 30.52%, respectively compared with the control value (27.11%) (Table 1 & Fig. 1). Lauric (C 12:0) and stearic acid (C 18:0) contents showed slight increase. lauric (C 12:0) was 1.042% in untreated sample and increased to 1.154, 1.138 and 1.106% when the cotton seeds were exposed to gamma irradiation doses of 0.5, 1.0 and 1.5 kGy respectively, while stearic acid (C 18:0) was 2.542% for the control and had slightly increased to 2.97, 2.79 and 3.214% when the cotton seeds exposed to doses of 0.5, 1.0 and 1.5 kGy, respectively. On the other hand, myristic (C 14:0) and arachidic (C 20:0) acids showed a slight decrease in their percentage in the irradiated samples compared to the control un-irradiated one.

Myristic (C 14:0) content was 3.105% in untreated cotton seeds, while decreased to 0.865, 0.898 and 0.899% when the cotton seeds treated by gamma doses of 0.5, 1.0 and 1.5 kGy, while, arachidic acid (C 20:0) was decreased from 0.9087% to 0.5427, 0.5153 and 0.5937% when the cotton seeds exposed to γ-irradiation doses of 0.5, 1.0 and 1.5 kGy, respectively (Table 1). Dowd *et al.* (2010) revealed that cotton seed oil contains palmitic and stearic in large amounts are more stable and they are naturally hydrogenated for purposes of keeping. Thus, gamma irradiation doses increase the oil preserving than untreated one.

The unsaturated fatty acids of palmitoleic (C 16:1) and oleic (C 18:1) were increased when the cotton seeds exposed to gamma doses as in (Table 2 & Fig. 1). The palmitoleic acid was 1.509, 1.612 and 1.654% if cotton seeds treated by gamma doses of 0.5, 1.0 and 1.5 kGy, respectively, compared with the control value (0.6547), while, oleic acid (C 18:1) percentage was increased from 21.98% to be 26.31, 26.37 and 27.94% at γ-doses of 0.5, 1.0 and 1.5 kGy, respectively. It is remarkable that the major constituent linoleic acid (C 18:2) decreased from 40.29% to be 33.59, 36.09 and 32.57% at gamma doses of 0.5, 1.0 and 1.5 kGy respectively (Table 2 & Fig. 1). This agrees with Rahimi and Bahrani (2011) who stated that linoleic acid content decreased by increasing irradiation doses. The oleic and linoleic content ratio agreed with the fact that there is a negative correlation between linoleic and oleic acid, that is, with increase in olic acid content the linoleic acid content decreases and vice virsa. This is expected since oleic acid is the precursor fatty acid for linoleic acid (Miquel and Browse, 1994, Ohlrogge and Browse, 1995, Shabnam *et al.*, 2011 and Topfer *et al.*, 1995). Cotton seed oil consists of 63% unsaturated fatty acids, including 22% mono-unsaturated (oleic) and 40% poly-unsaturated (linoleic), and 34% saturated fatty acids with 27% palmitic, can be described as naturally hydrogenated which make the oil stable for frying without the need for additional processing or the formation of trans fatty acids (Sekhar and Bhaskara, 2011). Moreover, Dowd *et al.* (2010) showed that the high content of linoleic (C 18:2) in cotton seed lower the oxidative stability than other vegetable oils and make the odour and flavour. In addition, the quality of cotton seed oil had not been seriously affected by either time or temperature of storage during the 2 year period.

TABLE 1. Effect of gamma irradiation doses on the cotton seed saturated fatty acids.

Fatty acid	**Non-irradiated** %	**Radiated** %		
	Control	**0.5** kGy	**1** kGy	**1.5** kGy
Lauric C 12:0	1.042[a] ± 0.033	1.154[a] ± 0.008	1.138[a] ± 0.0213	1.106[a] ± 0.003
Myristic C 14:0	3.105[a] ± 0.055	0.865[c] ± 0.004	0.898[c] ± 0.013	0.899[b] ± 0.012
Palmitic C 16:0	27.11[d] ± 0.068	32.48[a] ± 0.049	30.61[c] ± 0.076	30.52[b] ± 0.024
Stearic C 18:0	2.542[d] ± 0.016	2.97[b] ± 0.034	2.79[c] ± 0.007	3.214[a] ± 0.014
Arachidic C 20:0	0.9087[a] ± 0.013	0.5427[c] ± 0.010	0.5153[c] ± 0.003	0.5937[b] 0.003
Ts	34.707	35.0417	31.0613	33.4147

Ts: Total saturated fatty acids. Each value is the mean of three values ± Standard Error.

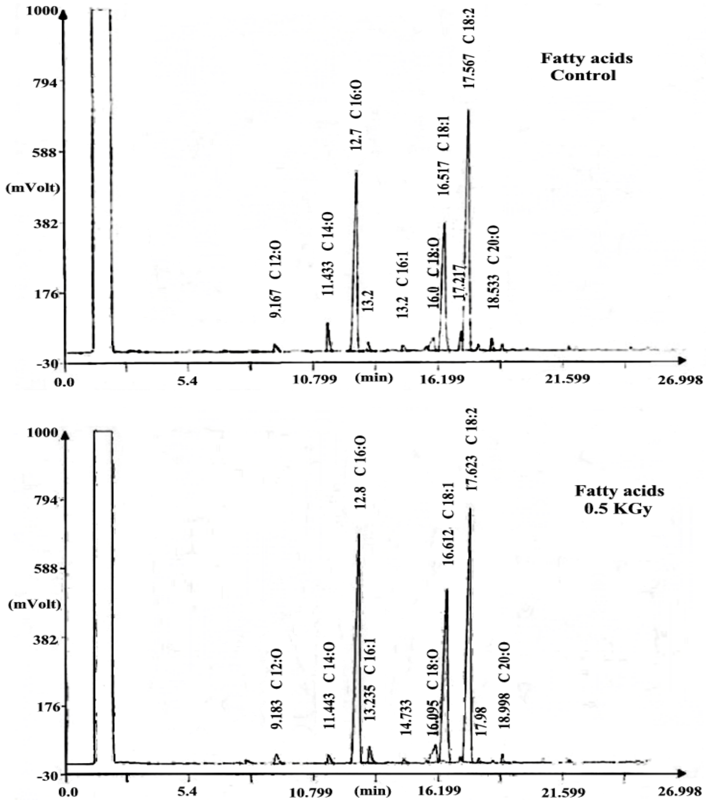
Many workers evaluated gamma radiation doses effects on some crop fatty acids, i.e Mexis and Kontominas (2009) evaluated cashew nuts' quality as a function of gamma irradiation dose in order to determine dose levels causing minimal undesirable changes to the product of cashew nuts at doses up to 7 kGy. Results showed fatty acids determined, stearic acid concentration increased; while, oleic acid decreased with irradiation dose. The irradiation indicating enhanced lipid oxidation.

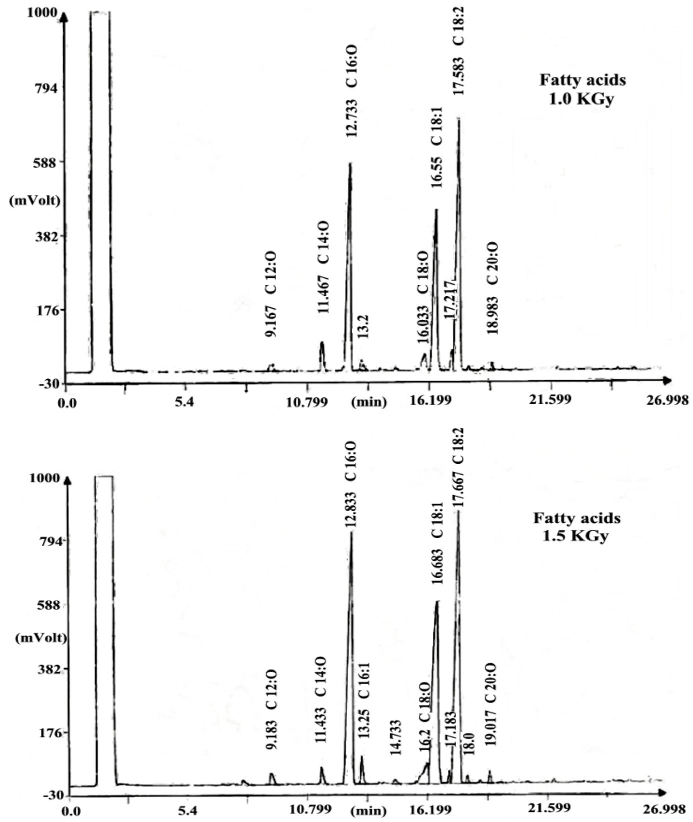
TABLE 1. Effect of γ- irradiation doses on the cotton seed unsaturated fatty acids.

Fatty acid	Non-irradiated %	Radiated %		
	Control	**0.5** kGy	**1** kGy	**1.5** kGy
Palmitoleic C 16:1	0.6547^{d} ± 0.004	1.509^{c} ± 0.006	1.612^{b} ± 0.005	1.654^{a} ± 0.003
Oleic C 18:1	21.98^{d} ± 0.058	26.31^{b} ± 0.036	26.37^{c} ± 0.031	27.94^{a} ± 0.023
Linoleic C 18:2	40.29^{a} ± 0.049	33.59^{c} ± 0.065	36.09^{b} ± 0.086	32.57^{d} ± 0.035
Tu	62.9247	61.409	62.382	62.164

Tu: Total unsaturated fatty acids. Each value is the mean of three values ± Standard Error.

Fig. 1. Chromatogram of saturated and unsaturated fatty acids as affected by gamma irradiation doses.





Effect of gamma irradiation doses on gossypol bound oil

The percent of gossypol in non-irradiated oil was 0.00357% (Table 3 & Fig. 2), but it increased slightly too; 0.00926, 0.01058 and 0.01114% when the oil irradiated as cotton seeds by γ-irradiation doses of 0.5, 1.0 and 1.5 kGy, respectively. Although gamma irradiation doses increase the gossypol bound oil, the increasing amount is not harmful to the human body as showed by (Hron *et al.*, 1987) who stated that some amount of gossypol tends to react with many natural substances in cotton seed and forms the bound gossypol that is non-harmful. However the unreacted gossypol known as “free gossypol” is toxic.

Gossypol still has toxins that decrease spermatogenesis and sperm motility in men. This is a topic that should be brought up with fertility doctors because cotton seed oil is a very commonly used ingredient in many foods Savanam and Bhaskara (2011).

Effect of gamma irradiation doses on β-sitosterol bound oil.

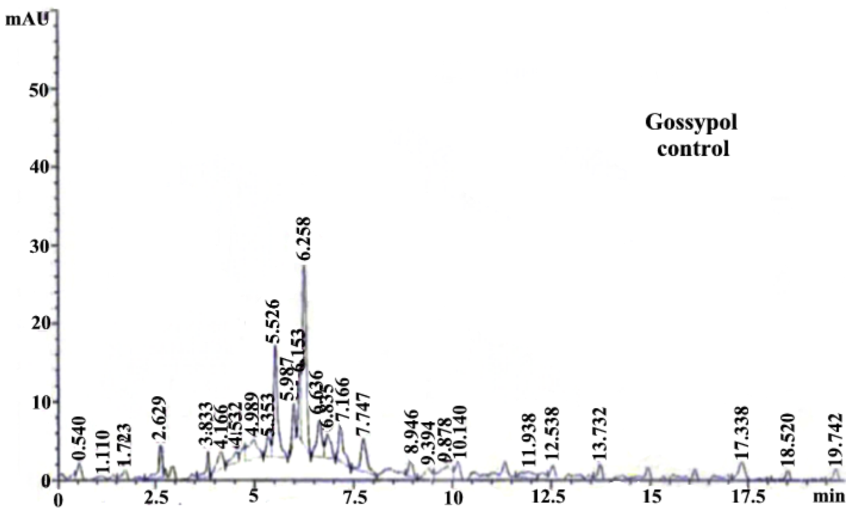
β-sitosterol content in cotton seed oil 0.1177% was found to increase by exposure to γ-irradiation. Although it increases in the three irradiation doses, 0.5 kGy is considered the best irradiation doses with a value of 0.1341% (Table 3 & Fig. 3). So, gamma irradiation doses especially dose of 0.5 kGy increase *β*-sitosterol in oil irradiated as cotton seeds. It increase the healthy valuable of oil because, *β*-sitosterol is known to control cholesterol levels, reduce the activity of cancer cell, promote prostate gland health and enhance immunity in the human body (Ye *et al.*, 2010).

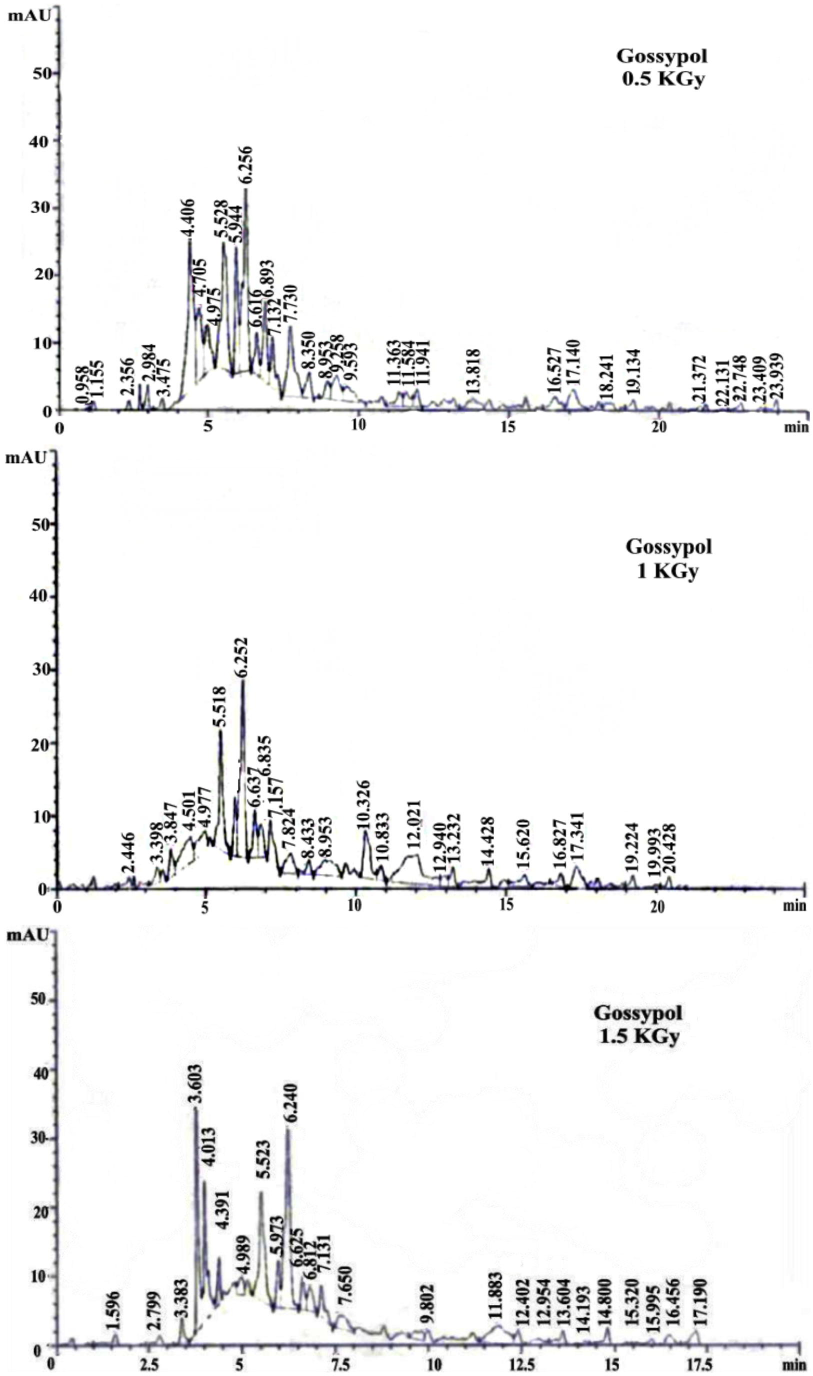
TABLE 3. Effect of gamma irradiation doses on the gossypol and *β*-sitosterol bounds oil in the cotton seeds.

Gamma doses (kGy)	**Gossypol** (mg/100g oil)	**Gossypol** %	***β*-sitosterol** (mg/100g oil)	***β*-sitosterol** %
Control	3.57[a] ± 0.032	0.00357	117.7[d] ± 0.154	0.1177
0.5	9.26[a] ± 0.040	0.00926	134.1[a] ± 0.124	0.1341
1	10.58[a] ± 0.046	0.01058	128.8[b] ± 0.119	0.1288
1.5	11.14[a] ± 0.035	0.01114	126.7[c] ± 0.115	0.1267

Each value is the mean of three values ± Standard Error.

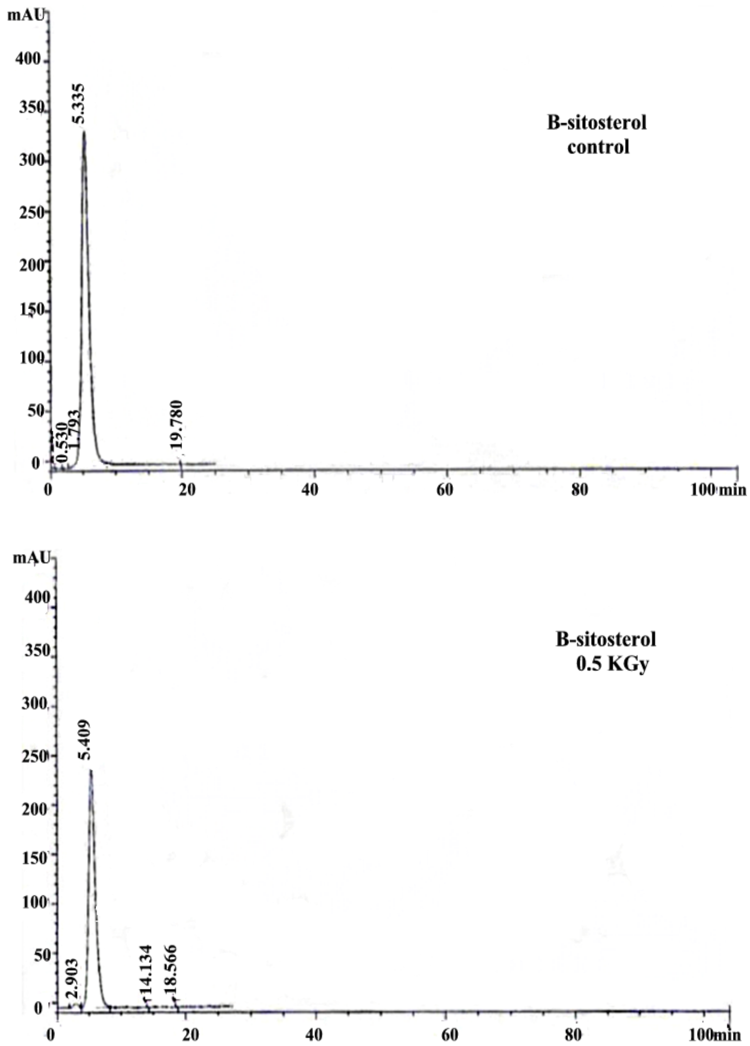
Fig. 2. Chromatogram of gossypol bound oil as affected by *γ-irradiation* doses.

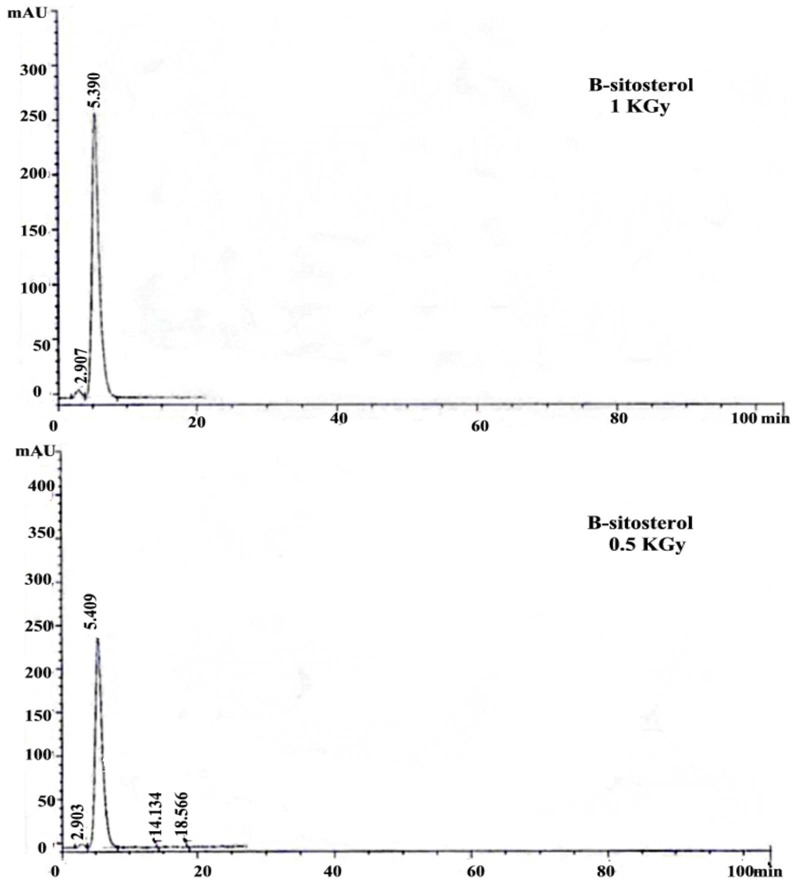



mAU
Gossypol
0.5 KGy
min
mAU
Gossypol
1 KGy
min
mAU
Gossypol
1.5 KGy
min

Generally, gamma irradiation dose of 0.5 kGy is considered the best dose enhance some oil chemical characteristics when irradiated as cotton seeds i.e. fatty acids of lauric, palmitic, palmitoleic, stearic, oleic that were naturally hydrogenated for long time preserve and antioxidants oil for long frying period. In addition, the same dose increase *β*-sitosterol content which improve the oil healthy and quality properties.

Fig. 3. Chromatogram of *β-sitosterol* bound oil as affected by γ-irradiation doses.





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تأثير جرعات أشعة جاما على بعض الصفات الكيميائية لزيت بذرة القطن

عمر إبراهيم صالح

قسم بحوث المنتجات الطبيعية ، المركز القومى لبحوث وتكنولوجيا الإشعاع ،
ص. ب. ٢٩ مدينة نصر ، مصر.

تم تعريض بذور القطن صنف جيزة ٨٥ (*Gossypium hirsutum* L.) لجرعات آشعة جاما ٠.٥ - ١ - ١.٥ ك.جراى وذلك بغرض تحسين بعض الصفات الكيميائية لزيت بذرة القطن مثل الأحماض الدهنية المشبعة وغير المشبعة والـ gossypol والـ β-sitosterol المرتبطة بالزيت.

وقد أشارت النتائج إلى ما يلى:

زادت الأحماض الدهنية المشبعة (Lauric - Palmitic - Stearic) فى زيت بذرة القطن التى تم تعريضها لجرعات آشعة جاما ٠.٥ - ١ - ١.٥ ك جراى. وعلى العكس من ذلك، حدث نقص فى الحامض الدهنى المشبع Arachidic فى جميع المعاملات الإشعاعية مقارنة بزيت بذرة القطن الغير معامل. كما زادت الأحماض الدهنى الغير مشبع (Oleic) فى عينات بذرة القطن التى عرضت للإشعاع مقارنة بالكونترول. وعلى العكس من ذلك يعتبر الحامض الدهنى الغير مشبع Linoleic أقل فى الزيت الناتج من البذور المعرضة للإشعاع عن البذور الغير معاملة.

زادت نسبة الـ gossypol والـ β-sitosterol المرتبط بالزيت فى بذور القطن تدريجيا بزيادة الجرعة الإشعاعية المستخدمة مقارنة بالكونترول.