

γ -Irradiation Effect on The Composition and Quality of Screw Pressed *Nigella sativa* (Black Cumin) Crude Oil during Storage

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NIGELLA *sativa* (black cumin) seeds were γ -irradiated with 10kGy, according to commercial practices, then screw pressed to extract the crude oil. The effect of γ -irradiation on the composition of the oil in terms of content, fatty acid composition, free fatty acids and total phenolics was studied. The effect of γ -irradiation on the quality of the oil during storage was also evaluated by monitoring the storage stability for a period of 280 days at ambient temperature (25°C) and in refrigerator (4°C). Results showed that γ -irradiation at 10 kGy increased the crude oil content from 32.7 % to 34.5%, total phenolics from 6.93 to 102.17 (mg GAE /g oil), and free fatty acids from 3.4 to 4.13 %. γ -Irradiation caused an increase in total saturated fatty acids from 20.40% to 21.45 %, accompanied by a significant decrease in total unsaturated fatty acids from 79.55 to 78.55%. The results also showed that exposing *Nigella* seeds to γ -irradiation at a dose of 10 kGy can affect the oxidative stability of the resulting oil during storage especially at room temperature. The storage stability of the crude oil can be enhanced by storing *Nigella* oil at 4°C. This data is relevant to dietary supplement industries for keeping the quality of crude *Nigella* oil by re-considering the dose of γ -irradiation applied for sanitizing the seeds.

Keywords: *Nigella sativa*, Crude oil, γ -Irradiation, Chemical composition and Storage stability .

The crude oil from the seeds of *Nigella sativa* (black cumin, Ranunculaceae) has been used to promote health and fight disease for centuries especially in the Middle East and Asia. The oil constitutes 34.0%-39.0% of the seeds weight⁽¹⁾ and has ~ 98.0-99.9% triglyceride oil fraction and the rest represents the volatile oil fraction⁽²⁾. The triglyceride fraction contains a high percentage (47.5%-61.2) of ω -6 linoleic acid and 18.9%-24.5% ω -9 oleic acid⁽³⁾. On the other hand, the volatile oil fraction of *N. sativa* is mainly thymoquinone (30.0%-63.0%) and p-cymene (18.0%-30.0%)⁽²⁾ as well as some minor phenolic compounds like thymohydroquinone, thymol and carvacrol. The volatile oil is reputed for its biological activity due to its content of these quinonic and phenolic

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constituents. These components justify the antioxidant⁽⁴⁾, anti-inflammatory⁽⁵⁾, anticarcinogenic^(6,7), antibacterial⁽⁸⁾ and antirheumatic⁽⁹⁾ activity of the volatile oil. The above mentioned nutraceutical and pharmaceutical properties of the crude oil of *Nigella* clearly explain its extensive use as a dietary supplement and in complementary medicine fields⁽¹⁰⁾.

Medicinal seeds and herbs are usually dried in the open air and are therefore prone to serious contamination by air- and soil-borne insects and pathogenic microorganisms which can diminish their biological activity. There has been a trend for γ -irradiation to become one of the most promising strategies for the sanitization of seeds and herbs to control this quality deterioration caused by microbial or pest contamination. This treatment attracted many countries during the movement to use "Atoms for Peace"⁽¹¹⁾. Toxicological tests confirmed the safety of γ -irradiation, especially at doses below 10 kGy without any deterioration of the nutritional value of the food⁽¹²⁾. This dosage was taken as the maximum allowed overall average absorbed dose for dried aromatic herbs⁽¹³⁾. On the other hand, the U.S. Food and Drug Administration (FDA) set a limit for irradiation treatment of culinary herbs and seeds that must not exceed 30 kGy^(14,15).

There are several studies concerning the efficiency of γ -irradiation in sanitizing various medicinal herbs^(16,17) including *Nigella* seeds⁽¹⁸⁾. However, more studies regarding *Nigella* are still needed to evaluate the effect of that treatment on the quality of the crude oil extracted from the γ -irradiated seeds, especially as it relates to long term storage.

Thus the current study was dedicated to study the effect of exposing *Nigella* seeds to the highest permissible dose (10 kGy) of γ -irradiation, on the chemical composition of the extracted crude oil. The quality of the crude oil during storage at two different temperatures was also assessed. The high dose (10 kGy) of γ -irradiation was also chosen according to the "commercial practice" for sanitization.

Material and Methods

Materials

Mature dried seeds of *N. sativa* were purchased from a specialized herbal retail store located in Cairo, Egypt. Gallic acid and Folin–Ciocalteu reagent were purchased from Sigma Chemical Co., (St. Louis, USA). All the solvents and other chemicals used were of analytical grade.

γ -Irradiation method

Nigella seeds (5 Kg) were packed in sealed polyethylene bags which was irradiated at ambient temperature with a dose of 10.0 Kilo Gray (kGy) using IR 206 Cobalt irradiator type JS 9500 Nordion, Canada, located at Irradiation Technology Research Center, Cairo, Egypt. The dose was controlled by the exposure time of the bags to the source of irradiation. The uncertainty of the given dose was 2.2% and the dose distribution in the sample bags was 1.07 kGy.

Extraction of the crude oils

5kg of both plain and γ -irradiated *Nigella* seeds were subjected to screw pressing to get the crude oil using commercial scale screw press machine. The seeds were exhaustively pressed and the crude oils were filtered and received in dark glass bottles submerged in an ice bath. Precautions were made to prevent cross contamination between plain and γ -irradiated seed oils by starting the extraction process with the former followed by washing the screw press machine. The crude oils were stored in a refrigerator until use within 2 weeks.

Extraction of the volatile oils

Part of each crude oil was mixed separately with distilled water (1:5 w/v) and the volatile oil was extracted by hydro-distillation for three hours using Clevenger-type apparatus. Before the beginning of distillation, 1 ml of n-hexane was added on the surface of water in the side arm of the Clevenger apparatus to collect the condensed droplets of the volatile oil and decreasing its partition with water to preserve the yield. At the end of the distillation process, the hexane layer (containing the volatile oil) was collected from the side arm, dried and evaporated under a slow stream of nitrogen to remove the hexane. The volatile oil was weighed and its content (yield percent) relative to the weight of crude oil was calculated.

Crude oil content

Crude oil content was evaluated quantitatively after extracting the seeds exhaustively with n-hexane using a Soxhlet extractor as previously described⁽¹⁹⁾. Hexane was evaporated using a rotary evaporator (Buchi Rotavapor Switzerland) at 40 °C. The oil was dried over anhydrous sodium sulfate then placed in a vacuum oven until constant weight. The oil was kept at -20 °C until analysis.

Determination of fatty acid composition

The methyl esters of *Nigella* crude oil were prepared according to A.O.A.C. method⁽²⁰⁾. Determination of fatty acids composition in the methyl ester was performed using a Hewlett Packard HP 6890 gas chromatograph equipped with an FID detector. The fatty acid esters were separated on an INNO Wax capillary column (30.0 m x 0.530 mm i.d. x 1.0 μ m film thickness). The carrier gas used was nitrogen with a flow rate of 1.5 ml/ min. The injector and detector temperatures were 230 °C and 250 °C, respectively. Column temperature was programmed from 100 °C to 240°C at a rate of 10°C/min and held at that maximum temperature for 10 min. The quantification of the fatty acid constituents was reported as percent of total FID peak area. All values reported are mean of at least two injections from two different preparations of fatty acid methyl esters \pm S.D. The identification of the peaks was made as compared with chromatograms of standard fatty acids methyl esters kit (Sigma-Aldrich, St. Louis, USA).

Determination of total phenolics

Total phenolics were determined colorimetrically using the Folin–Ciocalteu reagent as previously described⁽²¹⁾. Absorbance was measured at 750 nm. Measurements were done using a UV – 1601 PC UV-visible spectrophotometer (Shimadzu, Japan). Total phenolics were quantified by a calibration curve obtained from measuring the absorbance of known concentrations of gallic acid and the results were expressed as milligrams gallic acid equivalents (GAE) per 100g oil.

Determination of oxidative stability

The oxidative stability of oil samples was followed during a storage period of 280 days at ambient temperature (25°C) and in a refrigerator (4°C). The parameters measured for assessing the oxidative stability included peroxide value (PV) and para-anisidine value (*p*-AnV), according to the method of A.O.A.C.⁽²⁰⁾ and the total oxidation value (Totox) according to a reported method⁽²²⁾.

Statistical analysis

All determinations, except otherwise mentioned, were obtained from triplicate measurements and results were expressed as mean \pm standard deviation. The data were analyzed using one-way ANOVA and least significant difference tests for the mean differences between plain and irradiated samples for all parameters. The Statistical Package for Social Sciences (SPSS) for Windows version (14.0) was used to analyze the data (SPSS Inc., Chicago, IL). Statistical significance was calculated at $p < 0.05$.

Results and Discussion

Crude and volatile oil contents

The crude oil of *N. sativa* was extracted in this study using a screw pressing machine typically used for extraction of this oil commercially. Radiation treatment resulted in a significant increase in the crude oil content on a seed weight basis (Table 1). Irradiated *Nigella* seeds showed slightly higher oil content (34.5%) than plain seeds (32.7%), which was in agreement with other studies⁽²¹⁻²³⁾. The increase in oil content may be due to the rupture or weakening of cell membranes of *Nigella* seeds. It could also be due to degradation of some high molecular weight components⁽²¹⁾. Similarly, it was reported that the total extraction yield in some medicinal herbs increased by 5–30% with a 10 kGy dose of γ -irradiation⁽²⁴⁾. On the other hand it was found that a 10kGy of γ -irradiation decreased the crude oil content of *Nigella* seeds compared with plain seeds⁽¹⁸⁾. However, these authors did not give any explanation of their finding. Other work on this issue indicated that γ -irradiation of *Nigella* seeds at 10.0 kGy did not affect the content of the crude oil which was calculated after extraction with the screw press machine⁽²⁵⁾. The difference between our result and that work could be due to the high efficiency of Soxhlet apparatus (used in our study) in the exhaustive and quantitative extraction of oil from the seeds.

TABLE 1. Effects of γ -irradiation on the composition of *N. sativa* crude oil .

	Plain seeds	γ -irradiated seeds
Crude oil content (%)	32.7 ^a	34.5 ^b
Volatile oil content (%)*	0.1 ^a	0.1 ^a
Free fatty acid (%)**	3.4 ^a	4.13 ^b
Fatty acid composition		
Palmitic	13.10 ^a	11.57 ^b
Stearic	3.15 ^a	3.57 ^a
Oleic	21.19 ^a	20.70 ^a
Linoleic	57.54 ^a	57.43 ^a
Linolenic	0.81 ^a	0.41 ^b
Arachidic	4.22 ^a	6.31 ^b
Total SFA	20.47 ^a	21.45 ^b
Total MUFA	21.19 ^a	20.70 ^a
Total PUFA	58.35 ^a	57.84 ^a
S/P	0.35 ^a	0.37 ^a
UFA	79.54 ^a	78.55 ^b
Total Phenolic***	6.93 ^a	10.17 ^b

Values having the same letters in each row are not significantly different ($P < 0.05\%$).

* based on the crude oil weight; ** determined as oleic acid .

SFA = total saturated fatty acids; MUFA = total monounsaturated fatty acids; PUFA = total polyunsaturated fatty acids;

S/P = saturated: polyunsaturated fatty acids ratio; UFA = total unsaturated fatty acids ;

*** mg gallic acid equivalent/100g oil .

The volatile oil content for both plain and γ -irradiated seeds was 0.1 wt% based on the crude oil weight which indicated no effect of γ -irradiation (Table 1). Generally, this value is considered as poor volatiles content compared with 1.8% in other study⁽²⁾. That may be attributed to genetic variation and the effect of environmental factors on biochemical and enzymatic reactions that generate the volatile oil in the seeds.

Fatty acid composition

The fatty acid composition of γ -irradiated and plain samples is shown in Table 1. Fatty acid methyl ester (FAME) analyzed by GC revealed that both samples were composed mainly of six fatty acids (palmitic, stearic, oleic, linoleic, linolenic, and arachidic acids. Linoleic acid (57.43%, 57.54%) followed by oleic (20.70%, 21.19%) and palmitic (11.57%, 13.1 %) were the major fatty acids of γ -irradiated and plain seeds, respectively, constituting about 88.0-91.0% of total fatty acids of both oils. It was observed that γ -irradiation caused statistically significant increase in total saturated fatty acids (SFA) from 20.40 to 21.45 % and a decrease in total unsaturated ones (UFA) 79.54 to 78.55% (Table 1).

For individual saturated fatty acids, palmitic acid showed a significant decrease after γ -irradiation while arachidic revealed a significant increase. This behavior may be explained by breaking of double bonds and formation of radicals after irradiation followed by molecular structure rearrangement⁽¹⁸⁾. On the contrary, it was reported that the fatty acid composition of wheat germ oil, which is chemically similar to Nigella oil, was not affected after γ -irradiation⁽²⁶⁾. That could be due to the very low exposure doses (0.25-1.0 kGy) used in their study compared to the current study (10.0 kGy).

Free fatty acid content (FFA)

As shown in Table 1. FFA (determined as oleic acid %) slightly increased upon γ -irradiation which could be due to cleavage of ester linkage of acyl-glycerols and release of fatty acids in the free form. This result was in agreement with other investigators⁽²⁶⁾.

Total phenolic content

Phenolic compounds are hydroxylated derivatives of benzoic and cinnamic acids which contribute to overall antioxidant activities of plant extracts⁽²¹⁾. Data in Table 1 showed significant ($p < 0.05$) increase in total phenolic compounds from 6.93 to 10.17 mg (gallic acid equivalent/100g oil) from plain and γ -irradiated seeds, respectively. This pronounced increase might suggest some induced degradation of large phenolic molecules to smaller ones which still reacting with Folin–Ciocalteu's reagent. This agrees with the increase in phenolic acids in irradiated clove bud and nutmeg seeds, probably due to degradation of tannins⁽²⁷⁾. Phenolics in almond skin extract⁽²⁸⁾ and tocopherols of lyophilized mushrooms⁽²³⁾ also increased after γ -irradiation due to changes in the conformation of the molecules⁽²⁹⁾. In contrast, γ -irradiation decreased the amount of total phenolics in dehydrated rosemary after a doses range between 10- 30 kGy⁽³⁰⁾. This difference may be due to the dose and temperature of γ -irradiation process, phenolics composition, plant type and the physical state of the sample⁽²¹⁾.

Oxidation stability

Oxidative stability of oils refers to their resistance to formation of primary oxidation products (*e.g.*, peroxides, hydroperoxides, conjugated dieneetc.) or secondary oxidation products (*e.g.*, aldehydes, ketones, short chain acids....etc.)⁽³¹⁾. There is a number of analytical techniques that are usually used to assess oxidative stability of oils and fats. However, the peroxide and the para-anisidine values are considered among the most widely used techniques⁽³²⁾. Results obtained from these evaluations are discussed in details:

Peroxide value (PV)

This parameter measures the hydroperoxide content which is a primary oxidation product of oils. Figure 1 showed that during storage, the peroxide value increased significantly ($p < 0.05$) with γ -irradiation at ambient temperature (25 °C) or in refrigerator (4 °C) compared with plain sample, which came in

agreement with previous studies⁽³³⁻³⁵⁾. However, at low temperature (4°C) the oxidation rate proceeds much slower leading to a lower peroxide value than that at 25°C. The increase of peroxide value of the γ -irradiated samples is due to interaction of γ -irradiation with unsaturated fat molecules triggering oxidation, dehydration, and polymerization reactions.

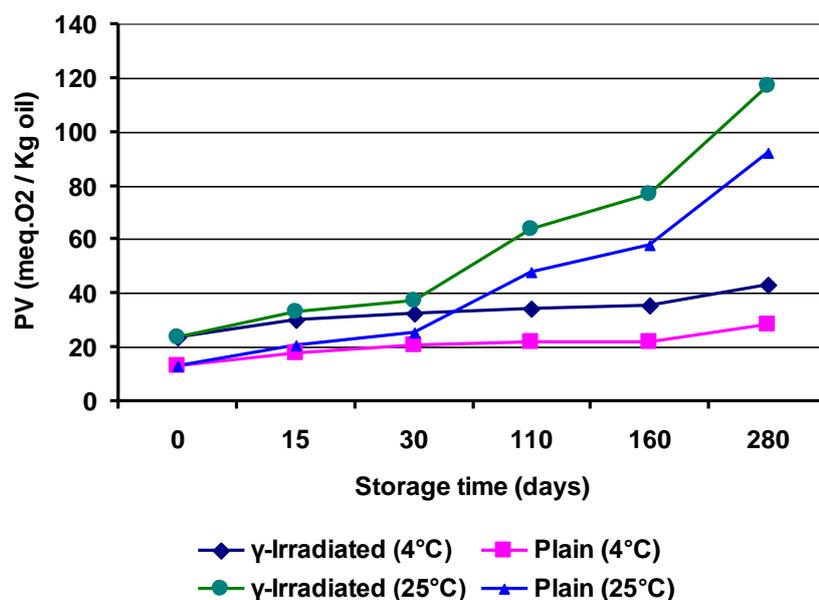


Fig. 1. Peroxide value of non-irradiated and irradiated *Nigella sativa* oil stored at 25°C and in refrigerator (4°C).

On the other hand, it was found that γ -irradiation decreased the peroxide value of almond skin extract⁽²⁸⁾. This finding was attributed to the breakdown of primary initial products of oxidation, including peroxides. The discrepancy in results may be interpreted mainly by differences in substrates, doses of irradiation, the presence or absence of pro- or antioxidants which is reflected on the steady state between the formation and degradation rates of peroxides.

Para-anisidine value (p-AnV)

This parameter measures the unsaturated aldehydes (principally 2-alkenals and 2,4-dienals) which are secondary oxidation product of oils. *p*-AnV is also an indicator of the oxidation history of the oil which means the previous oxidative damage that may happen to the oils, as these aldehydes are not volatile⁽³²⁾. Figure 2 revealed that at time zero, *p*-AnV of oil from γ -irradiated seeds was higher (30.83 mmol/kg oil) than that of oil from the plain seeds (16.13 mmol/kg oil). Unlike PV, *p*-AnV is increased and/or decreased very slightly in a plateau form throughout the entire storage period either at 25°C or

at 4°C, (Fig. 2). The increase in *p*-AnV due to γ -irradiation could be attributed to decomposition of hydroperoxides. *p*-AnV didn't increase proportionally with PV throughout the storage period, which revealed that formation of secondary oxidation products need longer oxidation time.

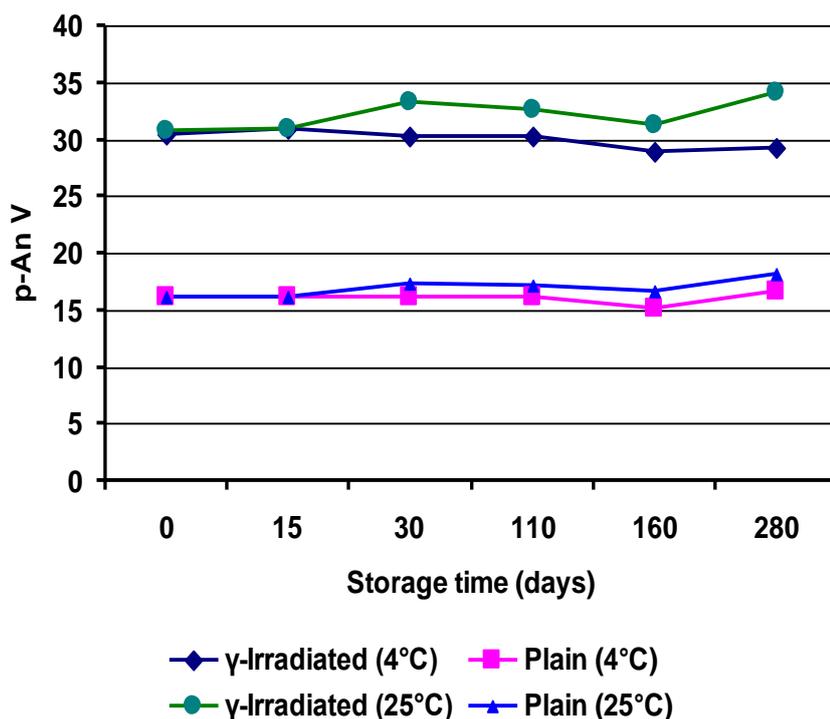


Fig. 2. *p*-Anisidine value of non-irradiated and irradiated *Nigella sativa* oil stored at 25°C and in refrigerator (4°C).

The total oxidation parameter (Totox value)

Oxidation is better assessed by the total oxidation parameter (Totox value). This parameter is considered a better index of oxidation than either PV or *p*-AnV alone⁽²²⁾. It is a combination of the hydroperoxides and their breakdown products ($Totox = 2PV + p\text{-AnV}$). Totox represents the quality, oxidation status and presence of degradation products formed from previous oxidation of oil or fat⁽³⁶⁾. Figure 3 showed that γ -irradiation of *Nigella* seeds greatly affected the crude oil that was stored at 4°C and 25°C. The difference in Totox value between plain and γ -irradiated samples was 37.0 % and 24.0% for storage at 4°C and 25°C, respectively. These differences reflect a significant effect of irradiation on oxidation stability of *Nigella* oil at different temperatures. Totox value of the tested oil samples also revealed the least oxidation of oils stored at 4°C throughout the entire period (280 days).

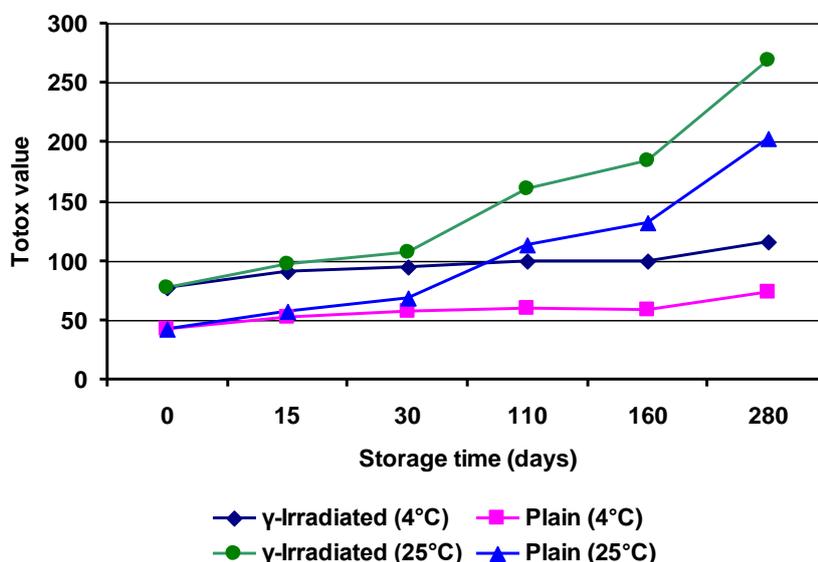


Fig. 3. TOTOX value of non-irradiated and irradiated *Nigella sativa* oil stored at 25°C and in refrigerator (4°C).

Conclusion

The quality of *Nigella* crude oil in term of composition and oxidative stability during storage was negatively affected by γ - irradiation of seeds at 10 kGy. However, storing the oil from γ -irradiated seeds at 4°C can retard the rate of oil oxidation to a great extent and help maintain a longer shelf life. Further research, including microbiological assay, is still needed to determine the least dose of γ -irradiation that induces minimum change to the oil quality during storage yet effectively controls microbial or insect growth on the seeds.

The data presented here is calling for re-considering the “commercial practice” dose of γ - irradiation (10kGy) in case of sanitizing *Nigella* seeds due to its negative effect on the quality of extracted crude oil.

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تأثير التشعيع بأشعة جاما على تركيب وجودة زيت حبة البركة الخام المستخلص بطريقة العصر الحلزوني أثناء التخزين

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في هذا البحث تم تشعيع زيت حبة البركة بأشعة جاما (10 كيلو جري) وفقا للممارسات التجارية ، ثم كبست البذور باستخدام المكبس الحلزوني لاستخلاص الزيت الخام. كذلك تمت دراسة تأثير أشعة γ -على تركيب الزيت من حيث الكمية ، وتركيب الأحماض الدهنية والأحماض الدهنية الحرة والفينولات الكلية. كما تم تقييم تأثير أشعة جاما على جودة الزيت أثناء التخزين أيضا من خلال رصد استقرار التخزين لمدة 280 يوما في درجة حرارة الغرفة (25 درجة مئوية) والتلاجة (4 درجات مئوية). وأظهرت النتائج أن التشعيع عند 10 كيلو جري سبب زيادة محتوى الزيت الخام من 32.7% إلى 34.5%، والفينولات الكلية من 6.93-10.17 ملجم مكافئ حمض الجالليك/جم زيت، والأحماض الدهنية الحرة من 3.4 حتي 4.13%. أظهرت الدراسة أن التشعيع قد أدى أيضا الى زيادة في مجموع الأحماض الدهنية المشبعة من 20.40% إلى 21.45%، يقابله انخفاض في إجمالي الأحماض الدهنية غير المشبعة 79.55 حتي 78.55%. كما أظهرت النتائج أن تعريض بذور حبة البركة لأشعة جاما بجرعة 10 كيلو جري يمكن أن تؤثر على الاستقرار التأكسدي للزيت أثناء التخزين وخاصة في درجة حرارة الغرفة. ويمكن تعزيز ثبات الزيت الخام ضد الأكسدة الذاتية عن طريق تخزين الزيت عند درجة 4 درجات مئوية. هذه البيانات تعتبر ذات الصلة بالصناعات الغذائية للحفاظ على نوعية الزيت الخام من خلال إعادة النظر في جرعة الإشعاع المستخدمة في تعقيم البذور.