Effect of different doses of gamma irradiation on biochemical and microbiological properties of sesame (*Sesamum indicum* L.) seeds

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Abstract

Seed irradiation during storage processes is one of the most effective methods to improve the seeds quality. This investigation was carried out to determine the effects of the different doses of ionizing radiation on biochemical and microbiological properties of sesame seeds and oil. To determine these effects, seeds were exposed to radiation with different doses 3, 6, 9 and 12 kGyto time period of 207, 413, 620 and 826 min respectively. The results showed that gamma irradiation had no significant($p>0.05$) effect on the different parameters. The ionizing radiation has no negative effect on the biochemical and microbiological quality of seeds and sesame oils. On the contrary, irradiation at different doses preserves the properties of sesame throughout the duration of storage While, smalldifferences, but sometimes significant ($p<0.05$), on protein and sugar contentswere recorded between irradiated and non-irradiated samples. The samples irradiated with the different doses (3, 6, 9 and 12kGy) preserved a great antioxidative activity, a high phenolic, flavonoids and protein content during the 12 months of conservation. Also, gamma radiation has ensured great stability throughout the experiment with regard to acidity and peroxide index. While, for the microbiological parameters, all the samples treated with the different doses remained completely free of bacteria at the end of storage period (after 12 months).

***Keywords: Key words**: biochemical properties; Gamma irradiation; Microbiological parameters; Sesame seed;, doses.*

1. Introduction

Sesame seeds, belonging to the Pedaliaceae family, are one of the oldest oil seed crops, grown for its edible seeds and oil, which present an important place in human nutrition, medicinal, pharmaceutical, industrial uses [1], [2]. Sesame has a high stability and resistance to oxidative deterioration. This resistance is due essentially to the high amount of lignin compounds (1034 ppm) such as sesamol, sesaminol and α-tocopherol [3] which is why sesame oil is used as cooking oil or seasoning and margarines. Sesame oil is known also by containing nearly 85% unsaturated fatty acids especially high contents of essential linoleic and linolenic acids which make a good food source. Sesame oil is manufactured by solvent extraction from raw sesame seeds followed by the refining process, or by pressing sesame seeds without refining [4].Sesame with other oleaginous seeds are considered as a good food sources, used extensively in the pharmaceutical and cosmetics industries due to its presumably low antigenicity [5], but are exposed to a major variety of contaminants, and microorganisms during cultivation and storage presenting a huge factor could be responsible of food deterioration [6]. It was recently reported that sesame could be a cause of allergic reactions among infant and children in Israel [7], and also a young woman in Italy [8]. It's thus important to monitor the growth of sesame and his cultivation, storage and transformation to prevent ulterior deterioration. During storage, the seeds and food products deteriorate rapidly [9,10] resulting a substantial loss in terms of vigor and germinability occurring particularly in humid condition, also, the interactions between fungal pathogen and seeds are higher during harvest period in humid conditions [11,12]. The food or seeds preservation involves the actions taken to maintain foods with the desired properties or nature for a desired time frame [13]. There's a need to preserve seeds to increase storage life using simple methods of preservation, storage and distribution which are some important factors in achieving food security. Food safety is the first attribute in food preservation followed by quality. The loss of quality depends on the type of food and composition, packaging and storage conditions [14]. There are different methods for the conservation of seeds and food, based on the mode of action. The methods could be categorized in either those inhibiting chemical deterioration and microbial growth like drying and freezing…, inactivating bacteria, yeast, molds, or enzymes like irradiation, sterilization….., and finally, avoiding recontamination before and after processing using hygienic processing or risk analysis and management [15, 16]. In recent decades, gamma radiation has been employed as an excellent tool for application in sterilization and preservation of food [17]. Ionizing radiation can safely and effectively eliminate the pathogenic bacteria from the food [18], disinfests the seeds, extend the shelf life of many products. The use of gamma radiation is based mainly on the fact that ionizing radiations damage the action of enzymes very effectively, deactivating living cells, thereby preventing microorganisms, insect gametes, from reproducing [19]. It is a non-thermal treatment with great high penetrating characteristics. Also, gamma radiation helps in preserving the quality of processed food compared to other techniques [19]. One of the products that encounters problems of deterioration when it is preserved is sesame. Our aim in this study was to investigate the effects of different doses of gamma irradiation on sesame seeds during long-time storage on biochemical and microbiological quality of seeds.

2. Materials and Methods

2.1. Irradiation treatment

Samples from sesame seeds were exposed to gamma radiation in a 60CO package Irradiator (INRA Tanger, Morocco). Irradiation was carried out in the stationary mode of operation with the possibility of varying dose rate (3, 6, 9, 12 kGy h-1) depending on the location and the distance from the source (10 to 40 cm). The samples were irradiated at place with a dose rate of 1.452 kGy/min. The irradiations were carried out at room temperature and atmospheric pressure. The radiation doses were measured with a dosimeter, the times of irradiation for the different doses are presented in

table 1, the seeds were stored for 12 months at room temperature in a gamma sterile air-tight polythene bag, the experiment was carried out for the irradiated and non-irradiated samples of sesame. the samples were divided into four groupsfor each dose of irradiation.

Table 1: Conditions of treatment of sesame samples by ionization

Debit (kGy/min)	Dose (kGy)	Duration of treatment (min)
1.452	3	207
	6	413
	9	620
	12	826

2.2. Biochemical analysis

For determination of the antioxidant activity of sesame extracts, the stable, 1 diphenyl-2-picryl hydrazyl (DPPH) radical was used [20]. The amount of total phenolic compounds was determined by using Folin-Ciocalteu reagent [21] and the flavonoidsamount was measured with the method of trichloridaluminium [22]. Total protein was determined by the method described [23]. Protein was extracted withphosphate buffer. After centrifugation, the supernatant was collected and the protein content was analyzed with the Bradford reagent. Determination of oil (fat), acid value (AV) (Oleic acid %) in term of mg KOH g-1 oil,peroxide value (PV) in term of mEqO2 kg-1 oil were determined according tostandard methods [24].

2.3.Microbiological analysis

Microbial load was determined using standard spread plate method (AOAC, 2010). The product of sesame seeds (10 g) was homogenized with 90 ml of sterile physiological water (9 g NaCl L-1). The homogenate then serially diluted and appropriate dilutions were plated on agar plate counts (APCs) (Bio-Rad 64475) for total bacteria counts (30 °C, 48 h),Sabouraud(Bio-Rad 64644) for fungi and yeast (30 °C, 3 or 5 days), lactose deoxycholate agar (Biokar 062HA) for total and faecal coliforms (37°C (Total coliforms) and 44°C (faecal coliforms), 24h) and Hektoen agar for Salmonella (37°C, 24h). After plating the colony forming units (CFUs) were counted, and microbial counts were expressed by mean of log CFU.

2.4. Statistical analysis

Statistical analyses were conducted using SPSS (Statistical Program for Social sciences) version 20.0 (Version 20, SPSS Inc., Chicago, IL, USA). All analyses were performed in triplicate and data reported as means ± standard deviation (SD).

3. Results and discussion

3.1 Effect of different doses of gamma irradiation on biochemical composition of sesame seeds

The conservation of food aims at preserving their edibility and their taste and nutritive properties. In particular, it makes it possible to prevent the growth of microorganisms and to retard the oxidation of fats which causes rancidity [25]. Common conservation methods are based primarily on energy or mass transfer, which aims to extend the shelf life of food products or to transform them through set of biochemical reactions or change of state [26]. The table 2 shows the biochemical properties the of irradiated and un-irradiated samples of cultivated sesame seeds. Generally,

sesame seeds have a low amount of moisture indicatesthat this seed is much easily preserved than those with high amount of moisture. The antioxidant potentials of sesame seeds were estimated from their power to trap the radical DPPH. The DPPH value of the irradiated seeds was higher than that of the non-irradiated control with 60.77% for the control and a mean of 66.80% in the first month of storage duration. The antioxidant activity of the un-radiated seeds begun to decrease until arrive to 46.5% in the end of the experiment, while for the irradiated samples the antioxidant activity remained constant with an average of 66.1% for the 12 months of storage. It is well- known that sesame has many functions for maintaining good health and it has been known for many years that sesame oil is highly resistant to oxidative deterioration, this remarkable stability is due to presence of a large quantity of endogenous antioxidants [27]-[28]. In this study, the irradiation treatment has preserved the antioxidant potential of sesame during the long conservation period. Phenolic compounds are distributed in the plant. They are an important antioxidant because of their ability to donate a hydrogen atom or an electron in order to form stable radical intermediates. Hence, they prevent the oxidation of various biological molecules [29]. In fact, several oilseeds and their by-products have been investigated for phenolic compounds in search for safe sources of natural antioxidants [30]. They play a role of defense in the case of external attacks and during a long storage against oxidation.

Table 2: Effect of different doses of irradiation on biochemical properties of sesame seeds

Treatment / **Storage period/month**	**Control**	**3 kGy**	**6kGy**	**9kGy**	**12kGy**
	Antioxidativeactivity%				
0	60,77±1,02	66,97±0,67	66,87±0,91	66,71±0,67	66,71±0,67
4	58,66±0,9	66,54±0,21	66,32±0,55	66,29±0,92	66,17±0,65
8	50,8±0,45	66,15±0,71	66±1,08	66,1±0,12	66,2±0,10
12	50,54±0,66	66,12±0,3	66,09±0,24	66,12±0,40	66,04±0,11
	Polyphénols totaux mg Eq galliqacid/g				
0	3,87±0,12	3,98±0,12	3,96±0,16	3,96±0,09	3,88±0,08
4	2,66±0,06	3,95±0,15	3,96±0,11	3,96±0,12	3,85±0,09
8	2,05±0,1	3,96±0,12	3,93±0,07	3,95±0,1	3,86±0,17
12	1,86±0,09	3,90±0,11	3,91±0,17	3,89±0,12	3,86±0,07
	Flavonoids mg Eq Quercetin/g				
0	0,13±0,03	0,145±0,007	0,145±0,007	0,145±0,02	0,14±0,01
4	0,11±0,011	0,145±0,007	0,145±0,006	0,14±0,005	0,14±0,01
8	0,07±0,007	0,144±0,01	0,145±0,009	0,14±0,1	0,14±0,1
12	0,07±0,009	0,143±0,008	0,144±0,006	0,141±0,002	0,141±0,01
	Protein g/100g				
0	25,24±0,5	26,06±0,76	26,12±0,54	26,21±0,67	26,23±0,99
4	22,6±0,55	25,99±0,81	26,07±0,52	26,1±0,54	26,15±0,60
8	21,66±0,19	25,9±0,31	25,98±0,6	26±,66	26,1±0,45
12	20,25±0,54	25,89±0,91	25,99±0,7	26,02±0,91	26,08±0,55

The total phenolic compound was determined by the Folin-Ciocalteu phenol method, measuring the reduction of the reagent by phenolic compounds with the formation of a blue complex that can be measured at 760 nm. Also, the

flavonoids possess a broad spectrum of chemical and biological activities including radical scavenging properties. Such properties are especially distinct for flavonols.The analysis of bioactive molecules during storage shows a significant decrease for polyphenols and flavonoids for the control with a percentage of 25%. Table 2 illustrates the severe alteration of untreated sesame seeds. Levels of bioactive molecules in irradiated sesame seeds remained stable for both parameters ranging between 3.98- 3.96 mg Eqgalliq acid/g for the polyphenols and 0.145-0.141 mg EqQuercetine/gfor the flavonoids content, proposing irradiation as an alternative method and choice for the preservation of sesame seeds.There was a highcorrelation between the estimated phenolic contents and the DPPH values ($P<0.01$) for sesame seeds. The protein content of sesame seeds for the control (25.24 mg/100g) found in this study (Table 2) is very much important to that reported by Franc and Brazzaville-Congo with the value 20% [31,32] and similar to other reported results for different cultivated varieties of sesame with the range of 22.30 -34.41% [33, 34, 35, 36]. The value of protein content for the control decreases during the 12 months of storage until reach 20.25 g/100, compared to the irradiation technique which allowed to keep a high protein value ranging between 25.9 g/ 100g for 3 kGy and 26.1 g/100g for 12 kGy, we noticed that the that the high doses of irradiation allowed a good preservation of the protein rate more than 3 and 6 kGy. Irradiation seems to be the most suitable method for preserving the quality of sesame seeds for all the parameters studied. This stabilization may be related to the effect of irradiation, which affects the chemical bonds and therefore induces the discharge of low molecular weight soluble phenols. The effect of irradiation has been reported for gamma irradiated plants at different doses. A significant increase in the composition of the total polyphenols of *Fagonia arabica* compared with the non-irradiated control was observed after the application of doses between 1 and 10 kGy [37]. Similar results have been reported [38, 39]. Similarly, it was correlated this change to the activation of phenylalanine ammonia-lyase (PAL) biosynthesis as the first enzyme involved in the synthesis of phenolic compounds [40].

3.2Effect of different doses of gamma irradiation on biochemical composition of sesame seed oil

The oil content of the control of cultivated sesame seeds was found to be 56.87% in the beginning of the experiment and 56.8 % after 12 months of storage (Table 3). This oil content fell in the range reported for different cultivated varieties of sesame seeds (44-58%) [33,34, 36, 41, 42, 43, 44]. The oil content of the sesame seeds in the present study was found to exceed that of some common edible oils such as cotton seeds (22-24%), sunflower (30-35%), soybean (18-22%), rapeseed (40- 48%), pumpkin seeds (31.2-51.1%) and olive (12-50%) [45]. Therefore, the sesame seeds can be considered as a potential source of vegetable oil for domestic and industrial purposes. The differences in proximate composition of sesame seeds and oil from the different areas may be caused by the spices variation, climate change, analytical conditions involved during analysis, processing and environment conditions in which they are grown. the samples irradiated with the different doses studied (3, 6, 9 and 12 kGy) also showed a stability concerning the oil content throughout the duration of the preservation (12 months) with an average of 56.10%. We could say that gamma irradiation has no effects on oil content of sesame seeds, Similar finding were reported saying that gamma irradiation had no real effect on the moisture content of oilseeds [46]. Also, gamma irradiation at 5 kGy and 10 kGy had no effect on the moisturecontent of African oil bean seeds. Also, it was reported that irradiation up to 16 kGy could slightly reduce most of the vital nutritional value of irradiated stored sesame seeds [47]. The results of table 3 indicate that the acid value (AV) and peroxide value (PV), of sesame seeds oil in the oil extracted from non-irradiated control sesame seeds samples found to be 2.19 mg KOH g^{-1} oil, 1.86 meq O2 kg^{-1} oil, respectively in the first month of storage, and begin to increase until reaching 4.2 mg KOH g^{-1} oil, 2.47 meq O2 kg^{-1} oil after the 12 months of storage, indicating oxidation of sesame oil. While oils from irradiated sesame seeds showed stability for both studied parameters over the shelf life with an average of 2.22 mg KOH g^{-1} oil and 1.84 meq O2 kg^{-1} oil. Therefore, it can be said that the treatment

prevents thealteration of sesame oil. The results of the present study are in contrary with the results reported previously showing an increase in free fatty acids for oil extracted from gamma irradiated almond seeds [48], the increase may be due to slight random hydrolysis of triglycerol molecules to free fatty acids and diacylglycerols [49]. The oxidation of oils and fats is one of the main causes of the deterioration of organoleptic and nutritional characteristics of food stuff [50]. The ionizing irradiation might affect the quality of oils by increasing the oxidationrate. It may also produce active spices like free radicals, which initiate certainchemical reactions that might also result in rancidity of oil and fat [51]. But in this study, it was observed that irradiation has no negative effect on the quality of sesame seeds and oil during conservation. On contrary, this treatment preserves their nutritional and biochemical properties even with the long storage time.

Table 3: Effect of different doses of irradiation on biochemical properties of sesame seeds oil

Treatment / **Storage period/month**	**contrôl**	**3 kGy**	**6kGy**	**9kGy**	**12kGy**
	Oil content %				
0	56,87±1,12	56,6±1,33	56,66±1,17	56,64±1,32	56,7±1,8
4	56,76±1,86	56,71±0,95	56,65±0,94	56,7±1,09	56,71±1,07
8	56,8±1,99	56,61±1,9	56,61±1,34	56,75±1,54	56,66±1,8
12	56,8±1,33	56,51±2,18	56,16±1,07	56,47±1,19	56,02±1,92
	Acidity mg KOH/g oil				
0	2,19±0,15	2,01±0,14	2,19±0,18	2,09±0,21	2,17±0,11
4	3,65±0,17	2±0,12	2,14±0,19	2,1±0,21	2,09±0,13
8	3,98±0,21	2,09±0,12	2,14±0,22	2,13±0,16	2,16±0,23
12	4,2±0,13	2,11±0,16	2,16±0,2	2,2±0,21	2,21±0,29
	Peroxid m Eq O2/ kg oil				
0	1,86±0,15	1,71±0,10	1,75±0,17	1,76±0,15	1,78±0,14
4	1,89±0,17	1,75±0,25	1,74±0,09	1,78±0,08	1,76±0,09
8	2,01±0,19	1,8±0,17	1,81±0,14	1,79±0,22	1,8±0,19
12	2,47±0,22	1,85±0,13	1,87±0,05	1,80±0,16	1,85±0,09

3.3 Effect of different doses of gamma irradiation on microbial composition of sesame seed

The spread plate method and direct observation were used to evaluate the total bacterial loads, yeast, total and faecal coliforms and fungal loads of sesame seeds. The control sample of sesame seeds contained a relatively small population of total flora, total coliforms, faecal coliforms, and yeasts. This population increases with the conservation time (Table 4). Contamination of materials taken directly from nature depends on the available surface, so that flowers and leaves can be contaminated 100 times more than fruits and seeds [52]. After 12 months of storage and comparison with untreated sesame, total flora reached 4.02 10^3 CFU / ml, total coliforms 3.5 10^3 CFU / ml, faecal coliforms 2.97 10^3 CFU / ml and yeasts 3.05 10^3 CFU / ml. On the other hand, salmonella is present in the form of a negligible population. Irradiation was found to cause significant ($p<0.05$) reduction in total flora, total coliforms, faecal coliforms, yeasts and salmonella proportionate to dose delivered. The doses of 3, 6, 9 and 12kGy reduced the initialbacterial and fungal load to below detection level when analysis was carried outimmediately after radiation treatment and even with the 12 months of conservation. Samples treated with 3 kGy, or more remained completelyfree

of fungi and bacteria throughout the storage. Radiation treatment was explored as a possible means to achieve microbialdecontamination of the food products and to preserve to quality of seeds because it has been established as a safe non-thermal process which, in general, the difference in dose not affect the intrinsic physicochemical propertiesof the products [56]. Based on studies on botanicals [49, 53, 54, 55,52], it is possible to derive dose requirement for microbiologicaldecontamination.

Table 4: Effect of different doses of irradiation on microbiological properties of sesame seeds

Treatment / **Storage period/month**	**contrôl**	**3 kGy**	**6kGy**	**9kGy**	**12kGy**
	Total flora (UFC/ml)				
0	$1{,}97\ 10^{3}$	< 1	< 1	< 1	< 1
4	$2{,}88\ 10^{3}$	< 1	< 1	< 1	< 1
8	$3{,}96\ 10^{3}$	< 1	< 1	< 1	< 1
12	$4{,}02\ 10^{3}$	< 1	< 1	< 1	< 1
	Total coliforms (UFC/ml)				
0	$1{,}12\ 10^{3}$	< 1	< 1	< 1	< 1
4	$2{,}50\ 10^{3}$	< 1	< 1	< 1	< 1
8	$3{,}06\ 10^{3}$	< 1	< 1	< 1	< 1
12	$3{,}5\ 10^{3}$	< 1	< 1	< 1	< 1
	Fecalcoliforms (UFC/ml)				
0	$0{,}88\ 10^{3}$	< 1	< 1	< 1	< 1
4	$1{,}48\ 10^{3}$	< 1	< 1	< 1	< 1
8	$2{,}55\ 10^{3}$	< 1	< 1	< 1	< 1
12	$2{,}97\ 10^{3}$	< 1	< 1	< 1	< 1
	Yeasts (UFC/ml)				
0	$1{,}89\ 10^{3}$	< 1	< 1	< 1	< 1
4	$2{,}23\ 10^{3}$	< 1	< 1	< 1	< 1
8	$2{,}86\ 10^{3}$	< 1	< 1	< 1	< 1
12	$3{,}05\ 10^{3}$	< 1	< 1	< 1	< 1
	Salmonella (UFC/ml)				
0	< 1	< 1	< 1	< 1	< 1
4	< 1	< 1	< 1	< 1	< 1
8	< 1	< 1	< 1	< 1	< 1
12	< 1	< 1	< 1	< 1	< 1

The effect of irradiation has been attributed to ionizing radiation acting directly or indirectly on the DNA and inducing local modifications of the double helix. The interaction with the DNA leads to ionizations and excitations that produce direct modifications of the molecule. In addition, the interaction of ionizing radiation with water molecules causes the radiolysis of water which results in the formation of free radicals. These products, very unstable, in turn, form other

radical species or molecules (H_2O_2) that have a high reactivity with any nearby biological molecule (water, protein, DNA). Their interactions with DNA produce chemical modifications of the polymer, such as oxidation [57, 58, 59]. In addition, the increase in the dose of treatment causes the effect decontamination. Similar results are presented in the case of decontaminations plants (Menthae pip., Cynaraescol., Valerianae rad., Lepidium sativum, Brassica nigra L. Koch and leaves of lemon grass) [60,52]. In addition, microbicide activity, induced by polyphenols and stressful conditions due to vacuum storage, is observed in the case inoculated non-irradiated control thyme. Burt demonstrated the effect of the essential oil on themembrane cell of bacteria. Due to its hydrophobic nature, it penetrates the membranes cells and mitochondria by making them permeable and leading to leakage of cells [61]. The radioactivity results showed that there is no difference in concentration of uranium (238U) and thorium (232Th) in unirradiated and irradiated sesame. Therefore, there is no contamination by radioactivity following gamma radiation, except for the existence of natural radioactivity (Table 5).

Table 5: Measurement of radioactivity of sesame

Samples	ρ_G^{LR} (10^{-5}. traces. $cm^{-2}.s^{-1}$)	ρ_G^{CR} (10^{-5}. traces. $cm^{-2}.s^{-1}$)	C (^{238}U)(10^{-6} g/g)	C (^{232}Th) (10^{-6} g/g)
Non irradiated	0,9 ± 0,08	4,16 ± 0,22	0,22 ± 0 ,01	0,54 ± 0,03
Irradiated at 3 kGy	0,91 ± 0,07	4,22 ± 0,29	0,22 ± 0,02	0,55 ± 0,05
Irradiated at 6 kGy	0,93 ± 0,02	4,29 ± 0,25	0,24 ± 0 ,01	0,55 ± 0,06
Irradiated at 9kGy	0,93 ± 0,05	4,35 ± 0,27	0,25± 0 ,01	0,56 ± 0,02
Irradiated at 12 kGy	1,95 ± 0,08	4,32 ± 0,34	0,25 ± 0,02	0,57 ± 0,04

Oilseeds used as a source of very important food are exposed to a wide variety of contaminants and are considered to be the main factors of food deterioration, especially during storage and preservation. Sesame seeds are frequently exposed to microorganisms during drying, harvesting and storage as a potential source of contamination. The deterioration of sesame seeds by microorganisms reduces viability, favoring the production of aflatoxins, because of climate change, this contamination problem is the most serious agronomic practices and storage conditions. The methods used during of conservation are different but some of them have much more effects negatively on the microbiological and nutritional quality of the seeds. From the analysis of the results obtained, the irradiation with different doses or even higher is the most suitable method to effectively decontaminate sesame while maintaining its nutritional value. The latter can also be achieved with all thermal techniques with positive effect on the preservation of sesame seeds.

4. Conclusion

The present investigation reveals the influence of long term storage and variation in absorbed gamma dose range on sesame seeds, the study shows that sesame seed present a great source in protein, oil, phenolic content and a high antioxidative potential. The different doses of gamma irradiation help to preserve the biochemical and microbiological properties during storage for the 12 months.

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