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Effects of gamma ray irradiation on olive fruits quality, enzyme activities and issued oil

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ABSTRACT

The results described in the present work concern the study of changes in gamma ray irradiated olive fruit (Tunisian variety: "Chemlali") quality along the storage time processing and the quality of olive oil issued. The study focused on the changes related to microbiological and physico-chemical properties, as well as pectinase activities in olives after irradiation. We also have been interested in the final product quality after oil extraction. The results of non irradiated olives were presented for comparative purposes. Mature olive fruits were treated with 0.5, 1 and 1.5 kGy γ ray radiation. Olive fruits were then stored for one month. Irradiation at 1.5 kGy allows the almost total destruction of the total aerobic germs, yeasts and moulds. Concerning physico-chemical parameters, the γ ray dose level enhancement generated an improvement in water retention capacity and then decreased the rate of polysaccharide hydrolysis in olives. Moreover, the irradiation dose of 0.5 kGy induced an increase in pectinase activities and the improvement of the protein extraction yield. The γ ray irradiation of olive fruit seems to not decrease olive oil oxidative stability in the studied samples. Finally, γ ray radiation was able to improve the yield of extraction of the oil (from 20.85 to 22.7 %) and insaponifiable fraction as polyphenols and beta carotenes.

KEYWORDS

Extraction yield;
Gamma irradiation:
Olive conservation;
Olive oil quality.

INTRODUCTION

Olive oil is a typical Mediterranean product. In many olive producing countries, processing of olives is not well synchronized with harvest due to limited oil extraction capacities of the local industry[8,11]. Therefore, after harvest, olives might be stored several weeks before processing for oil extraction [9]. The most important oil deterioration occurs during the harvest and processing procedures. Olive fruit long time storage under relatively high temperature and humidity, and low aeration may provide favourable medium for growth of fungi and bacteria [14]. Oil extracted from these contaminated and / or damaged olives can be high in acidity, low in stability [9], and high in volatile acids that cause musty smell[11,14]. However, there is a need to improve olive safety and the shelf life of this product. Olive fruit treatment after harvest for better storage, can

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improve the quality of olive oil by limiting acidity, oxidation and toxic compounds content.

Food irradiation is an economically viable technology for reducing post-harvest losses in quality, extension of shelf life of perishable products, improvement of hygienic quality of foods and inactivation of food-borne pathogens and parasites [4, 5,13,15,16,17,18,20,21,22]. Nowadays, there are no published data relating to the use of ^{60}Co γ-irradiation for extension of olive fruit storage life. In this paper, we have examined the effects of different doses of irradiation on visible changes generally associated with postharvest deterioration (microbial and physical impacts), as well as on selected enzymes (pectinases) widely considered to play a role in the process of oil extraction and then on the quality of extracted oil.

MATERIALS AND METHODS

Raw materials and irradiation treatments

Tunisian Black olives (*Olea europea*) variety 'Chemlali' from Sfax region were exposed to γ ray irradiation at 0.5, 1 and 1.5 kGy doses at the rate of 23.56 Gy min^{-1} with D_{max}/D_{min} ratio 1.04 in the National Center of Nuclear Science and Technologies (CNSTN). The source activity used was, Cobalt 60, 40000 Curies. Untreated fruit (0 kGy) served as control. Twelve portions of 100 g olive fruits were taken in each dose. During irradiation, olive fruits were packed in polyethylene. The absorbed dose was monitored with Poly Methyl Methacrylate (PMMA) dosimeter (Red Perspex 4034). The fruits were treated until reaching more than 90% of olive fruits that received the target dose.

Microbiological analyses

Total aerobic, yeast and mould colony forming units (CFU) were determined by standard spread plate methodology using plate count agar (PCA) and Sabouraud. PCA plates were incubated at 30°C for 48h and Sabouraud plates were incubated at 25°C for 3-5 days.

Physico-chemical analyses

Water content was determined from difference of fresh and dry mass and expressed as percent. Five grammes from each replicate were placed in Petri dishes and weighed before placing them in an oven at 130°C for 2h until they reached a constant mass. Measurement of pH: Five grammes from each replicate were crushed in 30 ml of distilled water and pH values were determined by pH meter Consort C931.

Pectinase analyses

Exo-pectinolytic activities were determined by measuring the amount of reducing sugars produced. The amount of reducing groups is expressed as the number of micromoles of galacturonic acid liberated after one hour incubation at 50°C with 0.9% citrus pectin (Sigma, France). Protein content was estimated by the method of Bradford[2], using bovine serum albumin as standard. One unit of exo-pectinolytic activity was defined as the amount of enzyme that liberates one μmole of galacturonic acid per minute.

Oil extraction

Olive oil was extracted in the Tunisian National Oil Office at room temperature using a procedure to imitate the industrial process (Leroy-Somer). About 400 g of each sample were triturated. The oil extraction yield was measured.

Acid value

Acidity value was expressed as percent of oleic acid. To determine free acidity, 1 g of oil were added to 30 ml ethyl alcohol-diethyl ether (1:1 v/v) mixture and neutralized with 0,177M NaOH according to the COI method[12].

Peroxide value

Peroxide value was given in milliequivalents of active oxygen per kilogram of oil. Oil (1 g), weighed precisely, was added to 25 ml of an acetic acid-chloroform (3:2 v/v) mixture. A volume of 1 ml of a saturated KI solution was added to this mixture and the sample was stored in dark during 5min. Distilled water (75 ml) and starch paste (1 ml), as indicator, were then added to the mixture and the sample was titrated with 0.01N sodium thiosulfate to complete bleaching.

K_{270} and K_{232} extinction coefficients

K_{270} and K_{232} extinction coefficients were measured using a UV spectrophotometer (Genesys 5). Oil sample

(0.25 g) was placed into 25 ml volumetric flask. The flask was made up to volume with cyclohexane for spectrophotometry.

Phenol and beta carotene content determination

The phenol content was determined colorimetrically at 727 nm (Genesys 5 spectrophotometer) by the Folin-Ciocalteu reagent[7], using cafeic acid as standard for the calibration curve. Results of analyses made in triplicate were expressed as mg of cafeic acid per kg of oil. Beta carotene content was determined as follows: 1g of olive oil was weighed into a 20ml flask and petroleum ether was added up to volume. The spectrophotometric reading was made at 452nm (Genesys 5 spectrophotometer). Data were expressed as mg of beta carotene per kg of oil.

Fatty acid composition

The composition of fatty acid methyl esters was determined by gas chromatography performed on an Agilent 6890 series GC chromatographer system, equipped with a 30 m capillary column Agilent 19091N-133 having a 0.25µm film thickness. The fatty acid methyl esters identification was based on R_f of known standards. Injector and detector temperatures were respectively 220 and 280°C. Oven temperature was held at 150°C for 1min, then increased first to 200°C at 15°C min^{-1}, and second to 250°C at 2°C min^{-1}, and held for 5 min at the final temperature. Column pressure was maintained at 16.95 psi.

All experiments were conducted in triplicates and results were obtained as the average of the experiments under the same conditions.

RESULTS AND DISCUSSION

1. Olive quality evaluation

Microbial analyses

High quality olive oil is necessarily extracted from healthy olive fruits. The total germs number can give an indication on the state of freshness or the state of decomposition of the olives. Gamma ray irradiation at 1.5 kGy resulted in significant reduction in total aerobic, yeast and mould microorganism content, while the irradiated samples at 0.5 and 1 kGy still sparingly contaminated throughout 14 days of storage, compared to the control (Figures 1 and 2). Otherwise, between days 14 and 28, all samples showed an increase in microbial populations.

Gamma ray irradiation could be then proposed for olive treatment in order to increase their conservation time and their quality before oil extraction. The effect of this treatment on physico-chemical properties of the fruit and oil quality should, however, be studied.

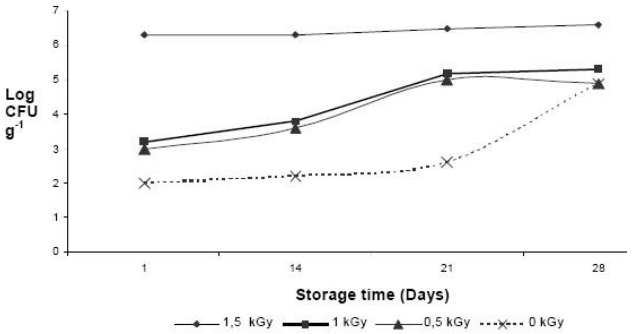

Figure 1: Effect of olive irradiation dose (γ rays) on the total aerobic contamination germs along one month storage time.

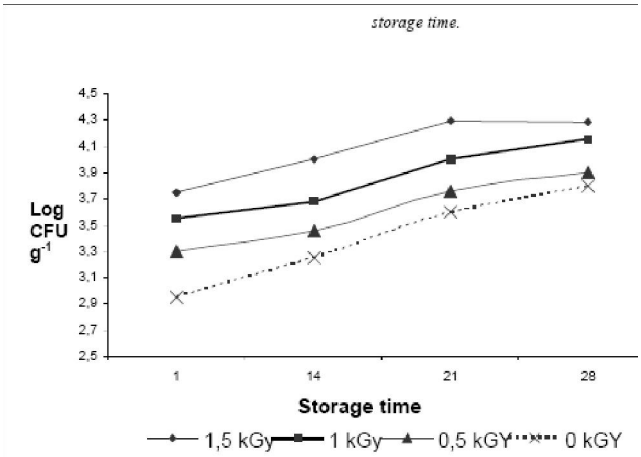

Figure 2: Effect of olive irradiation dose (γ rays) on the yeast and mould contamination germs along one month storage time.

Physico-chemical analyses in olives

pH and water content values can give an indication on the hydrolysis of polysaccharides composing olive cell walls. TABLE 1 shows that there was no significant change in pH values in olives either over storage time or as function of γ ray radiation dose. Also, olives conserve their water content after irradiation (TABLE 1). Higher water loss might be a consequence of fungal decomposition of olives resulting in the leakage of cell fluids[9].

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TABLE 1 pH and water content values over storage time of olive samples treated at different γ ray radiation doses.

Parameter		0 kGy	0.5 kGy	1 kGy	1.5 kGy
pH	Day1	5.42	5.51	5.41	5.42
	Day14	5.21	5.56	5.45	5.33
	Day23	5.49	5.04	5.77	5.26
Water content %	Day1	21.27	22.70	23.39	27.24
	Day14	19.99	22.05	23.14	27.50

Pectinase activities in olives

The effect of γ ray irradiation on pectinases was studied: These enzymes are described as involved in cell wall destruction in malaxation process. Gamma ray irradiation had no significant effect on the exo-pectinolytic activities compared to the control sample through day 1 of storage (Figure 3). However, differences between activities were found after day 7 of storage. The increase in pectinolytic activities can be correlated with the loss of firmness during storage and probably the development of microorganisms.

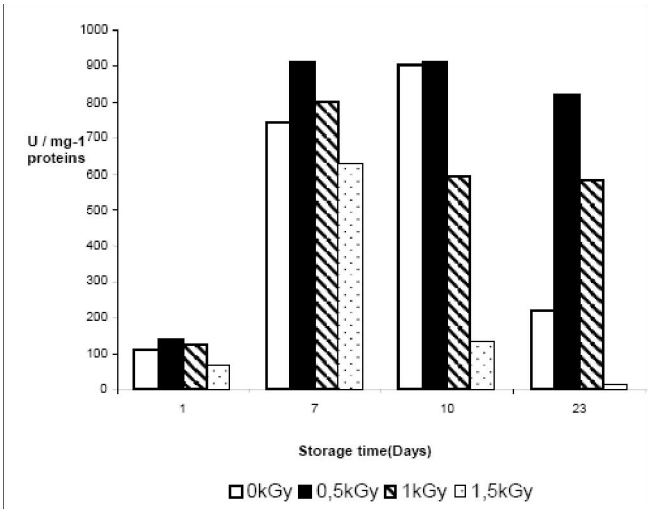

Figure 3: Variation of specific pectinase activities in olives as function of γ ray radiation dose and storage time.

2. Olive oil characteristics

Oil Parameters

Olive oil quality may be defined from commercial, nutritional or organoleptic perspectives[6]. The International Olive Oil Council[12] have defined the quality of olive oil, based on parameters that include free fatty acid (FFA) content, peroxide value (PV), UV specific extinction coefficients (K_{232} and K_{270}) and sensory score. In this section of the work we tried to check the absence of negative effect of olive fruit γ ray irradiation on the oil quality, in order to project an industrial application of γ ray irradiation. When studying the oil extraction yields, we observed an improvement of the olive oil extraction between 1.5 and 2% (TABLE 2). This rate is comparable to that obtained when using commercial enzyme preparations as processing aids to improve the extraction yield[19].

TABLE 2 Effect of γ ray irradiation dose on the olive oil extraction yield.

Irradiation dose	0 kGy	0.5 kGy	1 kGy	1.5 kGy
Oil Extraction yield (%)	20.85	22.7	22.3	22.3

TABLE 3 shows the influence of γ ray irradiation dose on the principal quality parameters. There was no significant change in free acidity value as function of γ ray irradiation doses. It remained below the limits reported by the International Olive Oil Council[12], (under 1 g of oleic acid per 100 g for a virgin olive oil).

The peroxide value is the main indicator of oxidation. The difference between irradiated and non irradiated samples was not significant up to 1 kGy γ ray irradiation dose. The peroxide value increases in oils extracted from olives treated at 1.5 kGy. However, peroxide value measured in all the treated samples was lower than 20méq of oxygen per kg of oil, which is accepted as the limit for extra virgin olive oil (TABLE 3).

After olive γ ray irradiation, no significant change was observed in UV absorbance of the extracted oils at 232 and 270 nm (TABLE 3). K_{232} value indicates the presence of conjugated bonds in oxygenated polyunsaturated fatty acids, whereas K_{270} is indicator of carboxyl compounds in olives[10]. The values were under the limits reported by the IOOC[12], and the differences between irradiated samples and the non irradiated one were non significant for both K_{232} and K_{270}.

TABLE 3 Influence of γ ray irradiation of olives on main quality parameters of the extracted olive oil.

Dose	0 kGy	0.5 kGy	1 kGy	1.5 kGy
Acidity (%)	0.21	0.11	0.18	0.18
pv meq d'O_2 kg^{-1}	4	6	6	9
K_{232}	1.002	1.014	1.014	1.070
K_{270}	0.048	0.05	0.047	0.100

These results suggest that the γ ray irradiated olives lead to oil that can be qualified as "extra virgin olive oil". The used physical treatment of the fruits seems to not affect the oil quality.

Polyphenols and Beta carotene contents

Total phenols and beta carotene contents increase gradually in oils extracted from olives treated at increasing γ ray irradiation doses (Figures 4 and 5). These concentrations were significantly improved in all the irradiated samples. This was may be due to an activation of antioxidant molecules after irradiation. Many studies report the very important role of those components as antioxidants in the oxidative stability of olive oil[1], contributing to maintain its shelf-life.

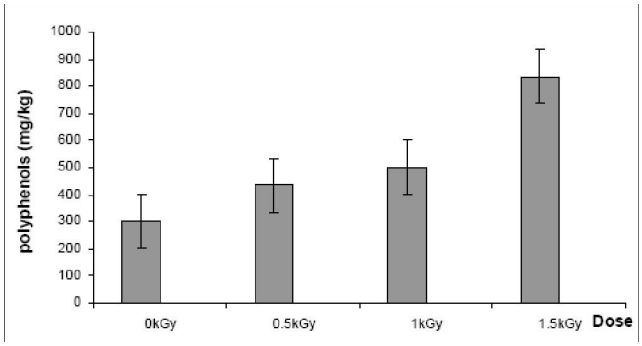

Figure 4: Variation of polyphenols concentration in oils under γ radiation of olive fruits before oil extraction processing.

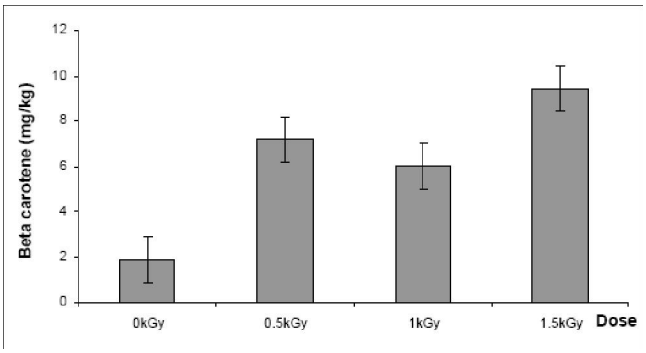

Figure 5: Variation of beta carotene concentration in oils under γ radiation of olive fruits before oil extraction processing.

Fatty acid composition

This section aims to observe changes in fatty acid content of oils as function of γ ray irradiation of olives. The results show that palmitic acid content was not significantly changed as function of the physical treatment (TABLE 4). Whereas, arachidic acid content declines from 1.02% at control test sample to around 0.5% after irradiation of olive samples (TABLE 4). Oleic acid, which content increased from 51.85 up to 54.77%, is the main fatty acid in olives treated or not by γ rays (TABLE 4). In general, there was no significant effect of irradiation on fatty acid composition.

TABLE 4 Influence of γ ray irradiation on fatty acids composition of the extracted olive oil.

Acid	0 kGy	0. 5 kGy	1 kGy	1. 5 kGy
palmitic (C16 : 0)	19.83	19.69	20.29	19.74
oleic (C18 : 1)	51.85	54.77	54.36	53.84
linoleic (C18 : 2)	23.79	22.75	22.31	23.2
linolenic (C18 : 3)	1.61	1.39	1.36	1.7
arachidic (C20 : 0)	1.02	0.46	0.54	0.48
eicosenoic (C20 : 1)	1.86	0.9	1.11	1.01

CONCLUSION

The microbial shelf life of irradiated olives was enhanced by γ ray irradiation. A 90% reduction in total aerobic contamination was reached at 1.5 kGy treated samples stored 14 days. Gamma ray irradiation effectively reduced total aerobic, yeast and mould populations through 14 days of storage. Softening of the irradiated olives was observed after γ ray irradiation at 0.5 kGy and higher. Gamma ray irradiation could be presented as efficient method for reducing microbial contamination of olives or other fruits for better storage before use or transformation. The importance of this treatment increases when observing the effects of olive irradiation on the physico-chemical properties in olives and the treated olive oil quality. In fact, we demonstrated in this work that few and non significant changes were observed in olives and oil after irradiation. We also observed an improvement in olive oil extraction yield and in anti-oxidant contents.

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