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The Effect of Gamma Irradiation on the Essential Oils and Antioxidants in Dried Thyme

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Abstract

This research was undertaken to investigate the effect of gamma rays at dose rates of 5.0, 10.0 and 15.0 kGy on the chemical composition of essential oils, total antioxidants, total flavonoids and total phenols, as well as the antioxidant activity and the thiobarbituric acid (TBA) and the free radical-scavenging activity (DPPH) of thyme. Radiation processing increased the total phenols, total flavonoids and total antioxidants of thyme, and moderate changes were detected at doses of 5 and 10 kGy for the essential oils. Thymol was sensitive to irradiation, especially at 15.0 kGy doses. In addition, the evaluation of antioxidant activity using DPPH radical-scavenging activity indicated some decreases of antioxidant activity in irradiated samples, while thyme exposed to doses of 10 and 15 kGy exhibited a significant increase in TBA values. The irradiation process can facilitate the utilisation of thyme as a preservative ingredient in the food and pharmaceutical industry.

Keywords: Irradiation; Thyme; Essential oil; Antioxidant; Flavonoid; Phenol

1 Introduction

The long history of herbs and spices has demonstrated their safe usage and excellent source for antioxidants. Thyme (*Thymus vulgaris* L.) is an herb descended from the *Lamiaceae* family. Thyme can be consumed as a whole spice/herb, or it can be ground, extracted, encapsulated or used as an emulsion. Thyme is characterized by its phytochemicals, efficacy as an antioxidant, and possession of phenolic compounds and flavonoids. Therefore, it has been determined to be a prominent herb from a medicinal and aromatic perspective (Embuscado, 2015).

Antioxidants are essential substances that inhibit other compounds from being oxidized (Aqil, Ahmad & Mehmood, 2006). Furthermore, the antioxidants produced by spices and herbs usually act with free radicals created in the initiation phase of autoxidation (Lee, Umano, Shibamoto & Lee, 2005). The antioxidants from thyme methanolic extracts can significantly prevent peroxidation in lipids (Fejes et al., 2000).

DPPH radical-scavenging activity is employed worldwide as an antioxidant activity assay; using this assay, a correlation between phenolic content and free radical-scavenging in nine plant extracts was observed. The chemical analysis of the extracts indicated the presence of phenolics, tannins, flavonoids, glycosides and alkaloids. The phenolic concentrations in dry plant extracts varied from 28.66 to 169.67 mg g^{-1} (Aqil et al., 2006).

Thyme's essential oils are distinguished by having a high content of important compounds, such as *thymol, carvacrol, y-terpinene* and *p-cymene.* These compounds range from 57.3% to 62.5% of the total oil content (Senatore, 1996). How-

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Nomenclature

TBA	Thiobarbituric acid number	FID	flame ionization detectors
DPPH	2, 2-diphenyl-1-picrylhydrazyl	GAE	Gallic acid equivalent
GLC	gas-liquid chromatography	IC 50	The half maximal inhibitory concentration

ever, it has been reported that thyme contains thymol and carvacrol in the 44-60% and 2.2-4.2% range, respectively, which can therefore be used to control lipid oxidation in foods (Alcicek, 2011). In addition, the prominent compounds quantitatively constitute approximately three-quarters of the total volatile compounds: *thymol* (72%) and *carvacrol (isothymol)* (5.7%) (Baranauskiene, Venskutonis, Viskelis & Dambrauskiene, 2003).

Although synthetic antioxidants can be used to prevent oxidation, they may not contribute additional nutritional benefits. On the contrary, the body can easily assimilate natural antioxidants, which are produced by spices and herbs, such as thyme (Embuscado, 2015).

Furthermore, thyme has long been used to preserve food and beverages via its phytochemicals. It has also been used as a natural food ingredient due to its colour and aroma (Alcicek, 2011).

Numerous aromatic substances have been generated from thyme extract. The extract consists of three acids, three aldehydes, four ketones and esters, seven alcohols, 14 aromatic compounds, 16 sesquiterpenes and 43 monoterpenes. In fact, the primary aroma in thyme is derived from phenolic compounds (Baranauskiene et al., 2003).

Food irradiation is a process involving the use of ionizing radiation, such as gamma rays, to generate various beneficial effects. The process can minimise post-harvest and storage losses, ensure hygienic quality, extend shelf life, simplify the trade of food products and enhance the parasitological and microbiological safety of foods by diminishing the effect of spoilage from microorganisms. Moreover, dried foods, herbs and spices can be exposed to ionizing radiation as an accredited preservation process (Nagy, Solar, Sontag & Koenig, 2011). Any of these effects depends on the radiation dose absorbed. Among all irradiated commercial products, irradiated spices and vegetable seasonings are the most commonly used. However, for commercial use, the typical dose is 10 kGy (Shurong, Meixu & Chuanyao, 2006).

Due to the importance of antioxidants in herbs and spices, Nagy et al. (2011) confirmed that radiation has no significant effect on their antioxidant properties nor on the division in the bonds of the glycoside. In fact, many types of herbs and spices exhibit a level of radioprotection even when consumed before radiation exposure (Farag, 2013).

The present study aimed to investigate the effect of gamma rays at dose rates of 5, 10 and 15 kGy on the chemical composition of essential oils, total antioxidants, total flavonoids and total phenols, as well as on the antioxidant activity of dried thyme (*Thymus vulgaris* L.).

2 Materials and Methods

2.1 Plant material

Samples of fresh aerial parts of thyme (*Thymus vulgaris* L.) were collected from a grocery market in Riyadh city, Saudi Arabia. The samples were prepared by washing and drying them in the shade. Then, 250 grams of plant material were placed in a series of polyethylene bags. For the chemical analysis, the bags were divided into groups; each sample had five replicates.

2.2 Irradiation process

A Gamma Cell was used for irradiation, delivering a dose rate of 14.2514 kGy h^{-1} at the time of the experiment. (Model Gamma Cell-220 from MDS, Nordion Initial, Canada: Activity source (Co-60) was 24,000 Ci of the production in December 1993) The Gamma Cell at King Abdul Aziz City for Science and Technology in Riyadh was used to expose the thyme samples, except the control sample, to 5, 10 or 15 kGy of gamma radiation.

2.3 Chemical analysis

After extracting the essential oils from the treated samples of thyme, the samples were analysed via gas-liquid chromatography (GLC).

2.4 Essential oil extraction

Each sample of dried thyme was placed in a flask filled with double-distilled water. Steam distillation was continuously applied for three hours until the oil was isolated; then, it was dried over anhydrous sodium sulphate (Pellegrini et al., 2003).

2.5 GLC analysis

Authentic essential oils were obtained from Dragoc (Holzminden, Germany) and were analysed using GC Pye-Unicam gas chromatography dual-flame ionization detectors (FID) with a chromate graph fitted with a coiled glass column (1.5 m x 4 mm) and packed with a 100-120 diatomitec mesh coated with 10% PEGA. The oven was programmed to gradually increase in temperature from 60 ^{o}C to 180 ^{o}C by 4 ^{o}C min^{-1}, and the isothermal process was continued for 15 minutes at 180 ^{o}C. The temperature for the detector was 220 ^{o}C, while that for the injector was 30 ^{o}C. The gas flow rate was 33 mL min^{-1} for hydrogen and 30 mL min^{-1} for nitrogen and air. After mixing the extracted essential oils with their main components, they were injected into the GLC to verify the resultant peaks (Jayaprakasha, Rao & Sakariah, 2002). For accuracy, the analysis was repeated five times.

2.6 Preparing samples for chemical analysis

Thirty grams of dried thyme was exposed to radiation in various doses and then weighed; they were then extracted by mixing them with distilled water, then stirring and turning them for 15 minutes, after which they were separated in centrifugal concentrators for 10 minutes (1000 × g). Afterwards, they were re-extracted several times and kept as an aqueous extract for subsequent tests. After filtering and extraction, 110 mL were obtained, and five replicates were made after each test analysis (Pellegrini et al., 2003).

2.7 Total antioxidant activity

The antioxidant content was estimated as equivalent to quercetin, which was used by Meda, Lamien, Romito, Millogo and Nacoulma (2005), by adding 0.75 mL of aqueous extract to 1.5 mL of a 2,2,-diphenyl-2-picryl-hydrazil (DPPH) solution in methanol at a 0.02-mg mL^{-1} concentration. Then, the mixture was left at room temperature for 15 minutes, after which the absorbance was read via a spectrophotometer with a wavelength of 517 nm and with 0.75 mL of water + 1.5 mL of methanol as a blank. The results were compared with similar cases when using the quercetin 6 mg mL^{-1} concentration.

2.8 Total phenolic assay

Determination the total phenolic content of thyme samples was accomplished using the Folin-Ciocalteu assay (Singleton & Rossi, 1965). One mL of the extract or a standard solution of gallic acid was added to 9 mL of distilled water in a 25 mL volumetric flask. A reagent blank was prepared using distilled water. The mixture was shaken after adding 1 mL of Folin-Ciocalteu phenol reagent. Then, 10 mL of 7% Na_2CO_3 solution was added to the mixture after 5 minutes. After incubating the mixture at room temperature for 90 minutes, the prepared reagent blank had a specified absorbance at 750 nm. One mg of gallic acid equivalent to GAE.100 g^{-1} of dried weight of thyme was used to express the

total phenolic content. For each determination, five samples were used (Meda et al., 2005).

2.9 Total flavonoid assay

An aluminum chloride solution (5 mL) was added to methanol at 2%, and the mixture was left for 10 minutes. Absorbance was read at a wavelength of 415 nm. A mixture of 5 mL of each methanol and the extraction was used as a blank. The results were then compared to quercetin at a concentration of 6.25 μg mL^{-1}. For each determination, five samples were used (Meda et al., 2005).

2.10 Radical scavenging activity

Antioxidant activity was estimated using the method of Meda et al. (2005), with some modification, i.e., the use of 2,2-diphenyl-1-picrylhydrazyl (DPPH). The formula used to calculate the activity was:

$$\%Inhibition = [(AC - AS).AC^{-1}] \times 100 \quad (1)$$

AC: the absorbance value of the control.
AS: the absorbance value of the test solution.
Subsequently, the half maximal inhibitory concentration IC_{50} was calculated by evaluating the DPPH radical scavenging activity at different concentrations of the water extracts.

2.11 Thiobarbituric acid value

The analysis was accomplished by the oxidative reaction products, which were determined as the thiobarbituric acid number (TBA). Two mL of trichloroacetic acid (20% aq.) and 2 mL of thiobarbituric acid solution (0.67% aq.) were added to 1 mL of thyme extract. The mixture was submerged in a boiling water bath for 10 minutes. Then, the mixture was cooled to ambient temperature before centrifugation of the 500 g mixture at 3000 rpm for 20 minutes. For each determination, five samples were used (Zin, Abdul-Hamid & Osman, 2002).

2.12 Statistical analysis

The data from the experiment were analysed using SAS system version 9.1.3. (Cary, NC), to calculate means, standard deviations and least significant differences. Results were expressed as mean ± (standard deviation) (SD) considering a P value of ≤ 0.05 as significant (Ott, 1984).

3 Results and Discussion

3.1 Essential oils

At low doses, the irradiation process is considered to be a cold, physical treatment for food because no significant heating occurs as a result of treating the samples. Therefore, irradiation has no direct effect on flavour compounds (Al-Bachir, 2016).
The important compounds were thymol (39.52 ± 0.15 mg .100 g^{-1}), *p-cymene* (21.60 ± 0.28 mg .100 g^{1}) and y-terpinene (18.41 ± 0.07 mg .100 g^{-1}), which constituted almost three-quarters of the total quantified volatiles, followed by monoterpenes, P-caryophyllene (2.71 ± 0.08 mg .100 g^{-1}), carvacrol (2.45 ± 0.07 mg .100 g^{-1}) and trans-sabinene hydrate (2.30 ± 0.14 mg .100 g^{-1}). Major volatile constituents, such as thymol, y-terpinene and carvacrol, were distinctive for *T. vulgaris* species and were also found as major compounds in other published results (Baranauskiene et al., 2003).
The results showed that radiation had significant effects on the concentrations of some compounds in the content of the dried thyme before and after different radiation doses. Table 1 shows the chemical composition of the essential oils of thyme after radiation at 5, 10 and 15 kGy compared with non-irradiated samples.
Thymol was stable when exposed to doses of 5 and 10 kGy (39.0 ± 0.165, 39.11 ± 0.087 mg .100 g^{-1}, respectively), yet its concentration decreased significantly when exposed to higher doses as compared with non-irradiated samples (38.50 ± 0.068, 39.52 ± 0.150 mg .100 g^{-1}, respectively). This result is consistent with the determination of 10 kGy as the commercial dose of dried spices, set by the World Health Organization World Health Organization (1988), needed

to reduce the loss of thymol, which is considered one of the most important compounds for inhibiting oxidation (Lee et al., 2005).
The concentrations of trans-sabinene hydrate were stable when exposed to doses of 5, 10 and 15 kGy (2.29 ± 0.224, 2.28 ± 0.035, 2.18 ± 0.012 mg .100 g^{-1}, respectively) compared with non-irradiated samples (2.30 ± 0.141 mg .100 g^{-1}).
The concentrations of *p-cymene* increased after exposure to a dose of 10 kGy (22.24 ± 0.230 mg .100 g^{-1}); a tendency towards reduction after exposure to a dose of 15 kGy (22.20 ± 0.135 mg .100 g^{-1}) was also shown. The lack, or small effect, of irradiation on the thyme aroma compounds is in agreement with Pereira et al. (2016).
The concentrations of y-terpinene decreased in samples exposed to a dose of 5 kGy compared with the control sample, while there was no obvious difference when exposed to doses of 10 and 15 kGy. There was also a reduction of carvacrol for all samples compared to the control, with the least-affected samples receiving a dose of 10 kGy. Generally, irradiation adversely affected the total content of the essential oils of dried thyme.

3.2 The total phenols, total flavonoids and total antioxidants

The results show the effects of Y-irradiation treatments on total phenols, flavonoids and antioxidants (Table 2).
The results illustrated that irradiated dried thyme at 5, 10 and 15 kGy had higher levels of phenolic compounds than the non-irradiated control sample in the methanolic extract. The significant increase in the phenolic content was 4954.67 ± 0.072, 5010.71 ± 0.015 and 4986.22 ± 0.075 mg .100 g^{-1} for the samples irradiated at 5, 10 and 15 kGy, respectively, compared to their content in the non-irradiated control (4925.73 ± 0.048 mg .100 g^{-1}). The highest total phenolic content occurred at a dose of 10 kGy.
The increase in the total phenolic content could be attributed to the inducement of a chemical reaction that decomposed the large molecules into small molecules, which are easily soluble in methanol and thus produce more solutes. This explanation is correlated with Huang and Mau (2006) findings. Moreover, Kim, Yook and Byun (2000) found that the total methanolic extract in 15 kinds of Korean medicinal herbs using various solvents increased by 5-25% at a dose of 10 kGy of γ-irradiation.
Gamma radiation causes an increase of soluble phenols in some spice extracts (Variyar, Limaye & Sharma, 2004). On the contrary, in some studies, no significant change was observed when comparing the phenolic content in the non-irradiated samples with that of irradiated samples at a dose of 20 kGy in *Agaricus blazei* (Huang & Mau, 2007), 30 kGy in *Rosmarinus officinalis* L. powder (Perez, Calderon & Croci, 2007), and 5 to 30 kGy in *Carum carvi* L. and *Laurus nobilis* L. (Polovka & Suhaj, 2010).
The total flavonoid content for thyme irradiated with a dose of 5, 10 and 15 kGy increased significantly by 43.66 ± 0.035, 47.42 ± 0.043 and 40.81 ± 0.039 mg .100 g^{-1}, respectively. Whereas in the non-irradiated sample, it was 36.41 ± 0.026 mg .100 g^{-1}. The maximum increase was obtained at the 10 kGy dose: 47.42 ± 0.043 mg .100 g^{-1}. However, these findings are in contrast to those of Zhu, Cai, Bao and Corke (2010), who reported a decrease in flavonoid content at a dose of 2 kGy and a minimum content at a dose of 10 kGy.
Total antioxidant content for thyme irradiated with 5, 10 and 15 kGy doses significantly increased, reaching levels of 2402 ± 0.880, 2419.66 ± 0.152 and 2408 ± 0.234 mg .100 g^{-1}, respectively. Whereas in the non-irradiated sample, it was 2392.25±0.027 mg .100 g^{-1}, with a maximum increase obtained at a 10 kGy dose, 2419.66 ± 0.152 mg .100 g^{-1}, as shown in Table 2. However, Taipina, Lamardo, Rodas and del Mastro (2009) reported no antioxidant content loss when irradiating pecan nuts with doses between 1-3 kGy.
The DPPH radical-scavenging activity and IC_{50} for irradiated thyme are shown compared with non-irradiated samples in Table 3.
The results indicate that the DPPH radical-scavenging activity of methanolic thyme extracts for an irradiated sample at doses of 5, 10 and 15 kGy were 0.58 ± 0.001, 0.56 ± 0.001 and 0.60 ± 0.003 % less than that for the non-irradiated control: 0.62 ± 0.001 %. A similar trend was observed in Abolhasani, Barzegar and Sahari

Table 1: The chemical composition of the essential oils of dried thyme irradiated with various doses of γ-irradiation

Compound	Quantity (mg.100 g^{-1} dry weight) Radiation Dose (kGy)				
	Control	5 KGy	10 KGy	15 KGy	*P*- Value
a-Thujene	1.61 ± 0.175	1.75 ± 0.156	1.99 ± 0.069	2.10 ± 0.025	0.00
a-Pinene	1.32 ± 0.109	1.90 ± 0.069	2.16 ± 0.089	2.01 ± 0.022	0.00
Myrcene	2.11 ± 0.077	2.15 ± 0.036	2.62 ± 0.168	2.60 ± 0.115	0.00
a-Teroinene	1.74 ± 0.139	1.99 ± 0.010	2.23 ± 0.154	2.33 ± 0.064	0.00
p-cymene	21.60 ± 0.282	21.90 ± 0.07	22.24 ± 0.230	22.20 ± 0.135	0.00
y-Terpinene	18.41 ± 0.076	18.21 ± 0.044	18.39 ± 0.048	18.36 ± 0.038	0.00
trans-Sabinene hydrate	2.30 ± 0.141	2.29 ± 0.224	2.28 ± 0.035	2.18 ± 0.012	0.482*
Linalol	1.42 ± 0.025	1.30 ± 0.052	1.21 ± 0.06	1.15 ± 0.018	0.00
Borneol	1.18 ± 0.01	1.22 ± 0.014	1.01 ± 0.01	0.98 ± 0.034	0.00
Thymol	39.52 ± 0.150	39.0 ± 0.165	39.11 ± 0.087	38.50 ± 0.068	0.001
Carvacrol	2.45 ± 0.079	1.14 ± 0.046	1.76 ± 0.063	1.55 ± 0.044	0.00
P-Caryophyllene	2.71 ± 0.08	3.55 ± 0.015	2.30 ± 0.041	2.13 ± 0.078	0.00
Total	96.37 ± 1.288	96.436 ± 1.20	97.33 ± 0.571	96.09 ± 0.104	0.212

(*) There was no significant difference between *trans-Sabinene hydrate* compound groups.
Values expressed as means ± SD (standard deviation). Repetition number = 5.
Values are significant differences (P $\leq$ 0.001).

Table 2: Total phenols, flavonoids and antioxidants of methanolic thyme extracts irradiated with doses of gamma radiation

Radiation Dose (kGy)	Contents (mg.100 g^{-1} dry weight)		
	Total Phenols	Total Flavonoids	Total Antioxidants
Control	4925.73 ± 0.048	36.41 ± 0.026	2392.25 ± 0.027
5 kGy	4954.67 ± 0.072	43.66 ± 0.035	2402 ± 0.880
10 kGy	5010.71 ± 0.015	47.42 ± 0.043	2419.66 ± 0.152
15 kGy	4986.22 ± 0.075	40.81 ± 0.039	2408 ± 0.234
P - Value	0.00	0.00	0.00

Values expressed as means ± SD. Repetition number = 5.
Values are significant differences (P $\leq$ 0.05).

Table 3: Scavenging activity and IC_{50} values of methanolic extract of thyme irradiated (10 mg mL^{-1}) against DPPH radicals

Radiation Dose (kGy)	% of DPPH scavenging activity	IC_{50} (mg mL^{-1})
Control	0.62 ± 0.001	0.27 ± 0.002
5	0.58 ± 0.001	0.46 ± 0.001
10	0.56 ± 0.001	0.51 ± 0.001
15	0.60 ± 0.003	0.37 ± 0.001
P - Value	0.00	0.00

IC_{50} value: the effective concentration at which the antioxidant activity was 50%; the (DPPH) radical was scavenged by 50%.
Values expressed as means ± SD. Repetition number = 5.
Values are significant differences ($P \leq 0.05$).

Table 4: TBA values of methanolic extract of thyme as a function of irradiation dose.

Radiation Dose (kGy)	TBA number (A_{532}) mean±SD
Control	0.77 ± 0.002
5	0.51 ± 0.002
10	0.98 ± 0.003
15	0.90 ± 0.003
P - Value	0.00

Values expressed as means ± SD. Repetition number = 5.
Values are significant differences ($P \leq 0.05$).

(2018), who found that DPPH activity decreased for irradiated pistachio green hull extracts at a dose of 10 kGy, then increased at a dose of 20 kGy. In contrast, there was an increase in the IC_{50} values of methanolic thyme extracts for the same doses, with the highest value recorded at a dose of 10 kGy.

A study conducted by Huang and Mau (2006) revealed that DPPH radical-scavenging activity exhibited no significant change as a result of irradiating freeze-dried mushrooms at doses from 2.5 to 20 kGy. Conversely, some studies reported an increase in DPPH radical-scavenging activity as a result of irradiating soybeans, green tea leaf extracts and rosemary leaf powder extracts at doses of 0.5-5 kGy, 10-20 kGy and 30 kGy, respectively (Jo, Son, Lee & Byun, 2003; Perez et al., 2007; Variyar et al., 2004).

Table 4 shows that exposing thyme to doses of 10 and 15 kGy gave a significant increase in the TBA numbers, which reached 0.98 ± 0.003 and 0.90 ± 0.003, respectively. On the other hand, at 5 kGy, the TBA number decreased to 0.51 ± 0.00 compared to the control sample, which was 0.77 ± 0.002. This result agreed with Suhaj, Rácová, Polovka and Brezová (2006), who studied black pepper methanolic extract irradiated at doses ranging from 5-30 kGy. Moreover, the current results agreed with Polovka and Suhaj (2010), who reported that the highest value for the TBA of irradiated caraway samples was achieved at a dose of 10 kGy.

An analysis of some irradiated edible and medicinal herb extracts at doses of 10, 20 and 30 kGy showed a change in the active components (Koseki et al., 2002). Under their experimental conditions, the only dose that had an inducement to the chemical substances of the extracts was at 10 kGy.

4 Conclusions

Radiation processing increased the total phenols, total flavonoids and total antioxidants of thyme, with moderate changes detected at doses of 5 and 10 kGy for the essential oils. Thymol was sensitive to irradiation, especially at a 15.0 kGy dose. In addition, the evaluation of antioxidant activity using DPPH radical-scavenging activity indicated some loss of antioxidant activity in irradiated samples, while exposing thyme to doses of 10 and 15 kGy significantly increased the TBA numbers.

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