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The Effect of Irradiation on the Quality Properties of Tarhana

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Featured Application: Irradiation is a useful application to preserve tarhana by preventing microbiological risks and pest formation. An irradiation dose of 5 kGy and below can be applied for the preservation of the tarhana without quality loss.

Abstract: Tarhana is a traditional food produced by the fermentation, drying and grinding of dough prepared with wheat flour, yoghurt, various vegetables and spices. Microbiological risks and pest formation are the major problems encountered during the storage of tarhana. In this study, the effect of irradiation was determined in order to eliminate microbiological risks and pest formation while preserving the quality features during the storage of tarhana. Depending on the irradiation dose, microbial inhibition occurred in tarhana samples, and the maximum protection was achieved with 10 kGy. Nevertheless, doses of 2.5 and 5 kGy inhibited the growth of *Bacillus cereus*. Additionally, all irradiation doses prevented pest formation. The consistency coefficient of soups prepared with irradiated tarhana samples decreased depending on the irradiation doses. There was no difference in 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity and total phenol content in the control with irradiated tarhana samples. However, the 10 kGy irradiated tarhana sample included higher thiobarbituric acid reactive substances. In conclusion, irradiation was applied for the first time to preserve tarhana by reducing the microbiological risk and preventing pest formation. Accordingly, a 5 kGy irradiation dose was recommended, with which the tarhana rheology was affected slightly.

Keywords: tarhana; food irradiation; microbiological safety; pest control; food safety

1. Introduction

Traditional fermented foods are a precious resource as they provide inexpensive, practical and convenient nutrients in the modern world under the threat of famine. Tarhana is a traditional fermented food that is prepared to consume as an instant soup by cooking a quantity of a dough powder in hot water [1,2]. It has nutritious and easy-to-digest properties, as well as having gained importance for infant nutrition in recent years [2,3]. Although there are slight differences in the production of tarhana, the tarhana dough is prepared by mixing wheat flour, yoghurt, sourdough and various vegetables and spices (tomato, red pepper, onion, mint, salt, etc.), and the dough is subsequently dried and ground after fermentation [1,4]. This fermented food is traditionally stored in a textile pouch, but plastic packages are preferred to avoid volatile loses [5]. However, it is difficult to keep the tarhana from moisture absorption, resulting in microbiological risks and pest formation problems during storage [6]. In fact, Turantas and Kemahlıoglu [7] showed the survival of the molds and some pathogenic bacteria such as *E. coli* O157:H7, *S. aureus*, *S. typhimurium* and *B. cereus* during the fermentation and storage period of tarhana. Additionally, aflatoxins ranging from 0.7–16.8 µg/kg were determined at 23.2% of 138 collected tarhana samples, whereas 29 samples contained Aflatoxin B1 (AFB1) ranging from

0.2–13.2 µg/kg [8]. Irradiation may be one of the possible solutions to reduce the risk of microbiological and pest encroachment during the storage of tarhana [9].

The use of irradiation [9–11], which involves the exposure of food to gamma and X rays to ensure quality and safety, has gained importance in recent years due to the large increase in foodborne diseases. Food irradiation is one of the prescribed methods for the control of the significant losses that can occur during the storage and distribution of products due to microorganisms, parasites and insects [9,11]. The high-energy rays applied to food systems stop the vitality of microorganisms or larvae in food by causing damage to their DNA. This sterilization of the food without the use of heat has led to the technique being referred to as "cold sterilization" [9]. The International Atomic Energy Agency (IAEA) concluded that energy beams emitted from food irradiated with doses below 60 kGy, with gamma rays from cobalt-60 and cesium 137, were less than 5 MeV in strength and could be considered insignificant [12].

The present study aims to determine the effect of irradiation on the quality and characteristics of tarhana to avoid microbiological risks and pest formation. Accordingly, the microbiological, chemical and physical effects of irradiation applied to packaged dried tarhana to prevent microbiological, physical and chemical losses during its storage is investigated.

2. Materials and Methods

2.1. Material

Five different flour type tarhana samples were purchased from local markets located in Denizli, Turkey in October 2015, which were blended and subsequently packaged in 5 kg plastic bags (LDPE, 30 × 52 cm, 60 micron thickness) for storage.

2.2. Irradiation and Storage Conditions of Tarhana Samples

The tarhana samples were irradiated by the GAMMAPAK Sterilization Ind. & Trd. Inc. (Tekirdağ, Turkey) with three different doses: 2.5, 5 and 10 kGy. Three packages of tarhana were irradiated for each dose, and three packages of tarhana were used as controls without irradiation. All the irradiated and control tarhana samples were stored in their packages at 25 °C for 5 months.

2.3. Microbiological and Pest Formation Analysis

The microbial analysis of tarhana samples (10 g) was carried out at the end of every month over 5 months of storage. The total aerobic mesophilic bacteria (TAMB) counts during the storage of tarhana samples were enumerated [13] on Plate Count Agar (PCA; Merck Co. Darmstadt, Germany) after 48 h incubation at 30 °C. The yeast grown in the tarhana samples was determined with a Dichloran Rose Bengal Chlortetracycline agar (DRBC; Merck Co., Darmstadt, Germany) at 25 °C after 5 days [14], and the *Bacillus cereus* was enumerated on *Bacillus cereus* agar (Merck Co., Darmstadt, Germany) supplemented with polymyxin B and trimethoprim at 30 °C after 72 h [15].

Pest analysis was performed by periodically observing the remaining material after 100 g of tarhana samples were sieved through a 2500 micron mesh during storage.

2.4. Acidity Degree, pH and Moisture Analysis

The acidity degree of the tarhana doughs was measured according to the TS2282 [5]. Accordingly, 10 g tarhana was homogenized with WiseStir mixer (HS-50A, DAIHAN, Korea) with 50 mL of 67% neutralized ethanol (Sigma-Aldrich, St. Louis, MO, USA). Then, the mixture was filtered (cellulose filter paper, Fisher Scientific, Loughborough, UK) and finally titrated with 0.1 M NaOH (Sigma-Aldrich, USA). The spent NaOH amount was multiplied by 5 to reach the acidity value of the tarhana dough samples. The pH value of the dough samples was determined as follows: 25 mL of distilled water was added to a 5 g sample and homogenized using a WiseStir mixer (HS-50A, DAIHAN, Korea). Distilled water was added to make a homogenous mixture of 50 mL, and the pH values were measured

using a pH meter (Eutech Instruments, Singapore). The moisture contents of tarhana samples during storage were measured according to AOAC (Association of Official Analytical Chemists) [16].

2.5. Color and Viscosity Analysis

The color properties of the tarhana samples were determined with using the Hunter-Lab Mini Scan XE color measurement device (Reston, VA, USA, ABD). In order to determine the viscosity values of tarhana, 10 g of tarhana sample was weighed and a 10% (w/v) tarhana–water mixture was prepared by adding 90 mL of pure water. This mixture was heated in a mechanical shaker and boiled for 20 min. The consistency coefficient (K) and flow behavior index (n) values of the tarhana–water mixtures were measured by a Brookfield programmable DV-II+ (Middleboro, MA, USA) viscometer. Samples were transferred to the sample container (Brookfield Accessories, SC4-13R) connected to a circulating water bath at 70 °C at 19 different speeds (5, 8, 10, 15, 17, 20, 25, 30, 35, 40, 50, 60, 70, 90, 105, 120, 140, 160 and 180 rpm) and K and n values were determined.

2.6. 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Radical Scavenging Analysis

The ability to scavenge the DPPH radical of tarhana samples was estimated by the methods of Wang et al. [17] and Fratianni et al. [18], respectively, with slight modifications. Aliquots of 0.1 mL tarhana extracts obtained from 1 g tarhana with 10 mL methanol (Sigma-Aldrich, USA) were mixed with 5 mL of 0.1 mM (prepared in methanol) DPPH radical in a test tube. The mixture was allowed to stand for 20 min at room temperature before the absorbance was measured at 517 nm spectrophotometrically (PG Instruments, Lutterworth, UK). The scavenging activity was calculated by the following equation:

$$\text{Scavenging activity\%} = ((\text{Absorbance of Blank} - \text{Absorbance of Sample}))/(\text{Absorbance of Blank}) \times 100$$

The "absorbance of blank" is the absorbance of the control reaction (containing all reagents except the test compound), and the "absorbance of sample" is the absorbance of the read test compound (517 nm).

2.7. Total Phenolic Content (TPC) Analysis

The tarhana samples were analyzed for total phenolic content by using the Folin–Ciocalteus assay after 1 g of tarhana sample was homogenized with 10 mL of methanol (Sigma-Aldrich, USA) and kept overnight for extraction at refrigeration temperature. The results were expressed in mg gallic acid (Sigma-Aldrich, USA) in 100 g of tarhana. [19].

2.8. Thiobarbituric Acid Reactive Substances (TBARS) Analysis

TBARS were determined using the extraction method described by Witte et al. [20]. TBARS numbers were calculated as mg of malonaldehyde per kg of tarhana (mg malondialdehyde/kg).

2.9. Statistical Analysis

All the experiments were carried out in triplicate. The microbiological, chemical and physical analyses of the tarhana samples, as well as the irradiation dose application, storage duration and intergroup differences, were determined a one-way analysis of variance using the MINITAB 17.1.0 program (State College, PA, USA).

3. Results and Discussion

3.1. Viable Organism Content of Tarhana Samples during Storage

In all irradiation doses applied, the formation of pests in tarhana samples could be prevented. However, in the control group without irradiation, a significant number of pests were found (Figure 1a). These results showed that irradiation was effective at preventing pest formation. The major problem

for dried tarhana during storage is pest formation, which is a spontaneous biological problem due to the development of larval eggs over time if the tarhana is stored under unsuitable conditions with high moisture and temperature. This problem is recognized to cause economic losses for many dried foods [3]. In this study, to test the effectiveness of irradiation, irradiated and control tarhana samples were observed at a constant high temperature (25 °C). Normally, it is advised that tarhana should be stored under 20 °C with low humidity [2,6].

The irradiation widely and distinctly reduced the microbial load. However, microbiological activity increased with extended storage time dependent on the irradiation dose applied, meaning that higher doses retarded the microbial growth for longer. The TAMB amounts of tarhana samples were closely related with the applied irradiation dose. The TAMB content at approximately 7 log CFU/gr for control samples was reduced substantially after irradiation (Figure 1b). However, the TAMB amounts significantly increased during storage ($p < 0.05$) for the 2.5 and 5 kGy irradiated tarhana samples. In 10 kGy irradiated samples, the TAMB counts remained below 1 log CFU/gr until the fifth month of storage. Similarly, the yeast mold content of tarhana samples was considerably reduced at all irradiation doses. Only the 2.5 kGy irradiated sample remained above 1 log CFU/gr. Although the yeast mold content of the 2.5 kGy tarhana sample reached the same level as the control group at the end of storage ($p > 0.05$), the amount of yeast mold remained below 3 log CFU/gr for the 5 kGy irradiated tarhana sample. The lowest amount of yeast mold was found in the 10 kGy irradiated sample (Figure 1c). After irradiation, the *Bacillus cereus* (BC) content was able to be reduced below 1 log CFU/gr at all doses applied. For the control tarhana sample, the BC content reached 5 log CFU/gr after 5 months. However, the BC content increased above 1 log CFU/gr after the first month of storage for both 2.5 and 5 kGy irradiated samples as well as after the third month of storage for the 10 kGy irradiated sample; however, the BC content was significantly below 2.5 log CFU/for at both irradiated tarhana samples (Figure 1d). These results indicate that irradiation is important for ensuring microbiological safety in tarhana. In addition, while a similar level of microbiological protection was provided in tarhana at 2.5 and 5 kGy doses, more effective protection was achieved in the 10 kGy irradiated tarhana sample. The effectiveness of irradiation is related with the microbial load of foods [9,11]. Indeed, tarhana is a fermented food which includes substantial amounts of lactic acid bacteria and yeast, although some of this flora are reduced during drying [1,2]. However, some spore-forming bacteria and molds could be contaminated according to the traditional drying conditions [6–8]. These microbiological results suggested that a minimum 5 kGy dose is required to eliminate the majority of fermenting and contaminating flora in dried tarhana.

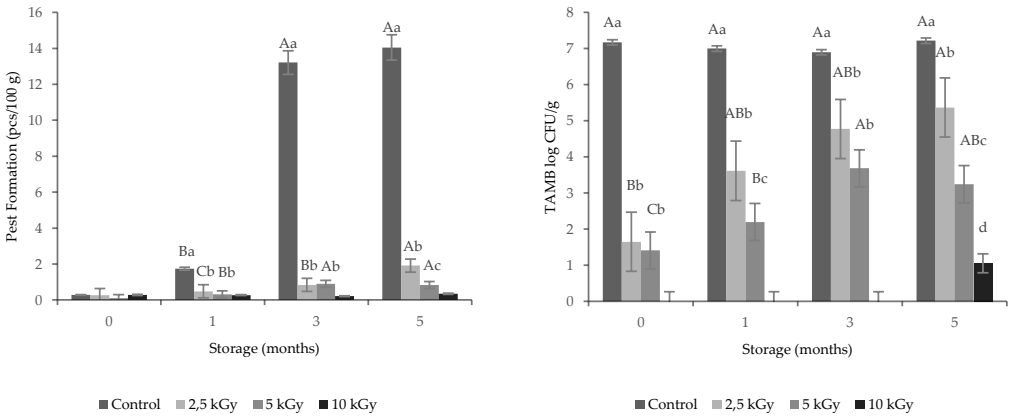
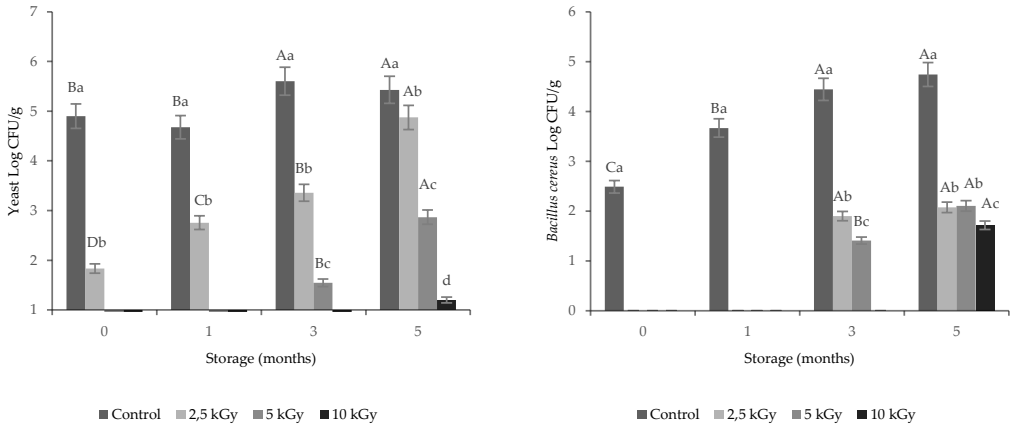


Figure 1. *Cont.*



(**c**) (**d**)

Figure 1. The microbiological changes and pest formation during the storage of irradiated and control tarhana samples. (**a**) Pest formation, (**b**) total aerobic mesophilic bacteria (TAMB), (**c**) yeast and (**d**) *Bacillus cereus* counts. Capital letters represent the $p < 0.05$ level of statistical difference between the storage times of the tarhana samples, while the lower-case letters show the $p < 0.05$ level of statistical difference between the irradiation doses.

3.2. Physical Changes in Tarhana Samples during Storage

The irradiation doses applied had a slight effect on the pH, acidity degree and moisture content of tarhana samples (Table 1). However, significant changes were observed in these parameters independent of irradiation with storage time. The pH of the tarhana samples increased during the storage ($p < 0.05$). There were no differences between tarhana samples at the beginning of storage, but the pH increase was slower ($p < 0.05$) in the 5 and 10 kGy irradiated tarhana samples for later storage times. In contrast to the pH increase, the acidity degree of the tarhana samples decreased during storage ($p < 0.05$); in particular, in the third month of storage, there was a significant decrease in the acidity degree of all tarhana samples ($p < 0.05$). There was no difference during storage for the acidity degree of tarhana samples with different irradiation doses. As expected, the moisture content of tarhana increased depending on the storage time; in particular, in the third month, the amount of moisture was increased significantly in the tarhana samples ($p < 0.05$). Moreover, the moisture contents of 5 and 10 kGy irradiated tarhana samples were lower from the beginning to the end of storage. Dried tarhana is hygroscopic and has a rapid moisture absorption ability [2,3]. Although tarhana is traditionally stored in pouches or commercial polyethylene bags [1,4], the pH increase and decreased acidity in addition to the increased moisture content might be the cause of microbial development and pest formation, as shown above.

Table 1. The pH, acidity degree * and moisture content changes during the storage of irradiated and control tarhana samples.

Samples	Months	pH	Acidity Value *	Moisture Content (%)
Control	0	4.28 ± 0.00 ^{Da}	23.25 ± 4.11 ^{Aa}	17.17 ± 0.05 ^{Ca}
	1	4.87 ± 0.28 ^{Ca}	18.40 ± 2.52 ^{Aa}	16.95 ± 0.24 ^{Bab}
	3	5.46 ± 0.57 ^{Db}	11.71 ± 0.82^{Aa}	18.44 ± 0.24 ^{Bc}
	5	6.16 ± 0.51 ^{Db}	11.40 ± 1.67 ^{Aa}	18.98 ± 0.15 ^{Bbc}
2.5 kGy	0	4.20 ± 0.00 ^{Ca}	20.92 ± 2.86 ^{Aa}	17.14 ± 0.21 ^{Ca}
	1	5.08 ± 0.16 ^{Ca}	17.20 ± 0.85 ^{Aa}	16.97 ± 0.25 ^{Ba}
	3	5.57 ± 0.03 ^{Cb}	12.45 ± 1.32 ^{Aa}	18.16 ± 0.42 ^{Bb}
	5	5.88 ± 0.56 ^{Cb}	10.43 ± 0.36 ^{Aa}	18.32 ± 0.36 ^{Bbc}

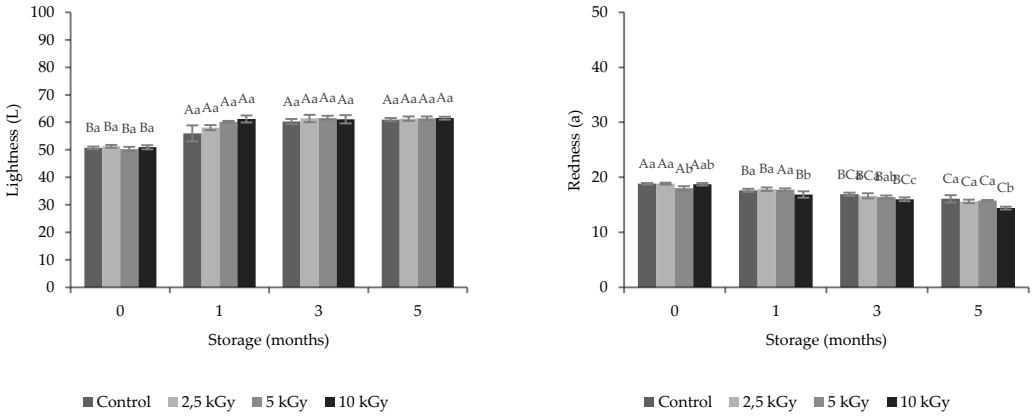
Table 1. *Cont.*

Samples	Months	pH	Acidity Value *	Moisture Content (%)
5 kGy	0	4.18 ± 0.01 ^{Ba}	22.57 ± 1.66 ^{Ba}	16.87 ± 0.13 ^{Ba}
	1	4.83 ± 0.04 ^{Ba}	19.35 ± 1.80 ^{Ba}	16.76 ± 0.21 ^{Aab}
	3	5.11 ± 0,03 ^{Bb}	12.33 ± 0.66 ^{Ba}	17.65 ± 0.22 ^{Ac}
	5	5.45 ± 0.14 ^{Bb}	10.70 ± 0.04 ^{Ba}	17.70 ± 0.38 ^{Abc}
10 kGy	0	4.15 ± 0.01 ^{Aa}	21.36 ± 0.59 ^{Ba}	16.98 ± 0.17 ^{Aa}
	1	4.54 ± 0.06 ^{Aa}	21.71 ± 1.01 ^{Ba}	16.64 ± 0.25 ^{Aab}
	3	5.21 ± 0.36 ^{Ab}	13.43 ± 0.46 ^{Ba}	18.09 ± 0.19 ^{Ac}
	5	5.68 ± 0.25 ^{Ab}	10.55 ± 0.62 ^{Ba}	18.36 ± 0.13 ^{Abc}

Capital letters represent the $p < 0.05$ level of statistical difference between the storage times of the tarhana samples, while the lower-case letters show the $p < 0.05$ level of statistical difference between the irradiation doses. *: The acidity values of the tarhana samples were given according to the standard TS8222 [5] due to the amount of NaOH used to neutralize the acidity of 10 g tarhana extracted with 67% ethanol.

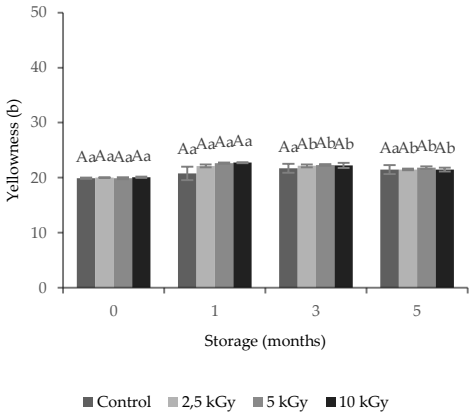
3.3. Color Changes in Tarhana Samples during Storage

The effect of irradiation on the color of tarhana is also of interest. In this sense, the effect of strong ionizing radiation should be evaluated in terms of the color components. The L value of the tarhana samples increased at the initial storage and stabilized after the third month. The rate of L value of tarhana increased depending on the irradiation dose applied, but there was no significant difference in the L value between the samples in terms of doses and storage time (Figure 2a). The whitening at the end of the first month observed in this study is a common problem encountered in the storage of tarhana. The main factor here may be the loss of color due to the effect of light. The "a" value slightly decreased during the storage. This might be related to oxygen or light contact rather than the effect of irradiation. However, the decline rate of the "a" value of tarhana samples was dependent on radiation dose, where the 10 kGy dose did reduce faster than the other doses (Figure 2b,c). In contrast, the "b" value of the tarhana samples increased in the first month and remained stable for a further four months. Tarhana has a characteristic yellowish color resulting from the paprika used in the ingredients, which is important for consumer perception [2]. These results clearly showed that irradiation has no detrimental effect on the color quality of tarhana samples, especially in terms of whitening, due to the loss of pigments arising from the paprika used in the production.



(a) **(b)**

Figure 2. *Cont.*



(**c**)

Figure 2. The color changes during the storage of irradiated and control tarhana samples. (**a**) Lightness L, (**b**) redness +a, (**c**) yellowness +b. Capital letters represent the $p < 0.05$ level of statistical difference between the storage times of the tarhana samples, while the lower-case letters show the $p < 0.05$ level of statistical difference between the irradiation doses.

3.4. Rheological Properties of Tarhana Samples during Storage

The consistency coefficients of soups prepared with tarhana powders decreased dramatically depending on the irradiation dose applied because the consistency coefficient of the unirradiated tarhana sample was higher than the irradiated samples during storage. However, 10 kGy irradiated tarhana samples were less viscous ($p < 0.05$) than other 2.5 and 5 kGy irradiated tarhana samples (Figure 3). However, there was no significantly consistent behavior between the 2.5 and 5 kGy irradiated samples. The consistency coefficient of unirradiated samples increased in contrast with the irradiated tarhana samples. Briefly, the irradiation destructively affected the viscosity of the tarhana samples in a dose-dependent manner. This might be related with the degradation of the chain structures of the amylose and amylopectin fractions of starch, which are mainly responsible with hydration in tarhana. It was commented that gamma radiation may contribute to molecular changes and the fragmentation of starch, leading to a progressive reduction in the molecular size of amylose and amylopectin by random cleavage of the glycosidic chains and consequently changes to the structural and physicochemical characteristics of starch [21]. However, the consistency coefficient of tarhana samples was in the range reported by Ibanoglu and Ibanoğlu [22] except for the 10 kGy irradiated tarhana sample.

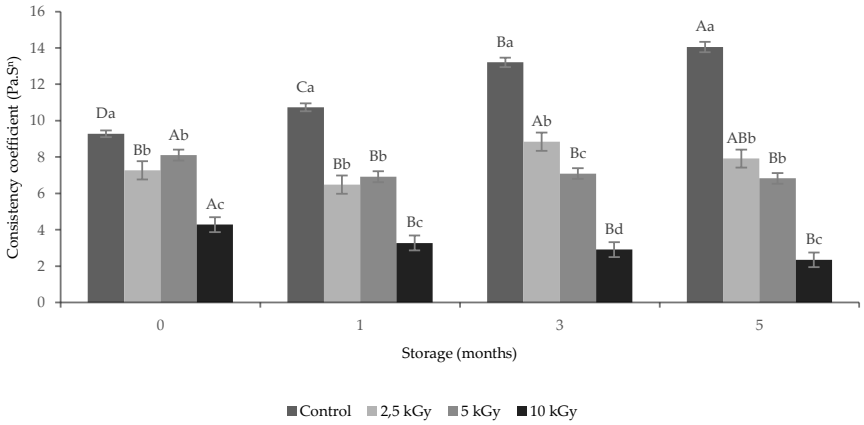
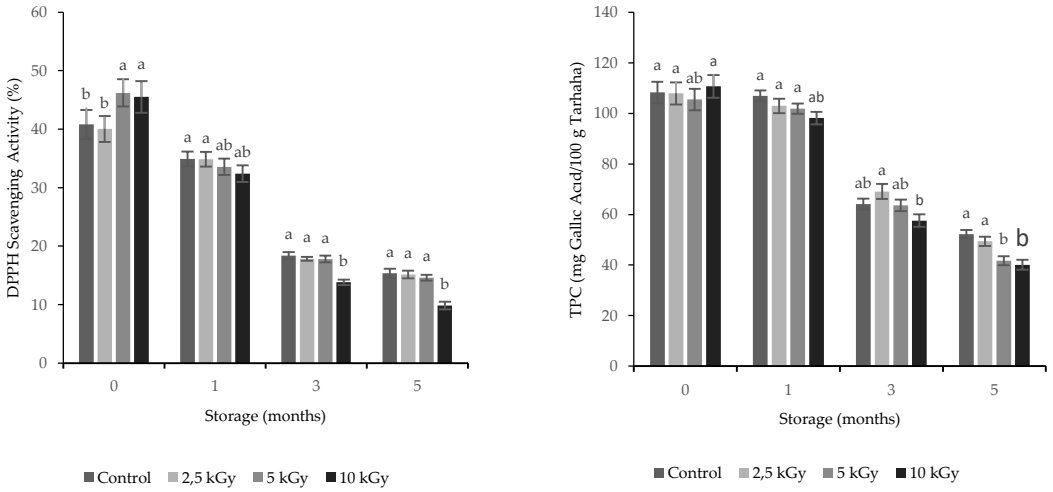


Figure 3. The consistency coefficient of soups prepared with using irradiated and control tarhana samples. Capital letters represent the $p < 0.05$ level of statistical difference between the storage times

of the tarhana samples, while the lower-case letters show the $p < 0.05$ level of statistical difference between the irradiation doses.

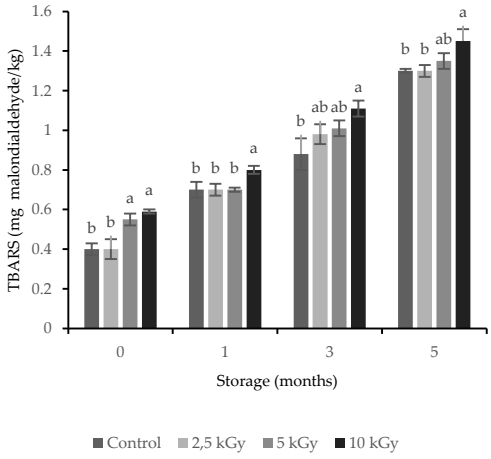
3.5. Antioxidant Properties of Tarhana Samples during Storage

The DPPH radical scavenging activity of tarhana samples, which were administered at 5 and 10 kGy irradiation doses at the beginning of storage, was found to be higher than that of the control and 2.5 kGy irradiated tarhana samples ($p < 0.05$). With continuing months of storage, no difference was observed between irradiated and control samples in terms of DPPH radical scavenging activity. However, the scavenging activity of tarhana samples decreased with the storage time; in particular, a significant decrease in scavenging activity was observed in the third month (Figure 4a). Similarly, there was no significant difference in the total phenol content between tarhana samples after irradiation and during storage. In the analyzes performed at the third and fifth months, only the 10 kGy irradiated tarhana sample had a lower total phenol content ($p < 0.05$, Figure 4b). As expected, the amount of TBARS in tarhana samples increased contrary to the decrease in antioxidant properties. After irradiation, the 5 and 10 kGy irradiated tarhana samples contained higher amounts of TBARS ($p < 0.05$). During storage, the amount of TBARS in tarhana samples increased and more TBARS were detected in 10 kGy irradiated tarhana sample each time (Figure 4c). The major concern regarding the adverse effect of irradiation in food systems is that it can trigger oxidation [9,11]. When evaluated in this respect, it was seen that irradiation did not cause a decrease in the antioxidant capacity of tarhana. Even at the beginning of storage, higher levels of antioxidant properties were observed. This might be due to the effect of irradiation resulting in the release of the antioxidant substances. On the other hand, irradiation did not significantly affect oxidation in tarhana samples. Higher oxidative products were formed only when 10 kGy doses were applied.



(a) **(b)**

Figure 4. *Cont.*



(c)

Figure 4. The antioxidant properties and changes of irradiated and control tarhana samples during storage. (**a**) 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, (**b**) total phenolic content (TPC), (**c**) thiobarbituric acid reactive substances (TBARS). Capital letters represent the $p < 0.05$ level of statistical difference between the storage times of the tarhana samples, while the lower-case letters show the $p < 0.05$ level of statistical difference between the irradiation doses.

4. Conclusions

In this study, the use of irradiation for the preservation of tarhana was evaluated for the first time. Irradiation was successfully applied to reduce microbiological risks as well as pest formation in tarhana during storage. Although irradiation showed no adverse effect on the color and antioxidation properties of tarhana, it reduced the consistency coefficient of the soups prepared from tarhana. Accordingly, instead of the 10 kGy dose, it is recommended to use 5 kGy or lower doses in order to preserve the quality features of tarhana.

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Conflicts of Interest: The authors declare no conflict of interest.

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