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Microbial profile of gamma irradiated thyme; cold prepared meal

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Abstract

The objective of this study was to investigate the effect of gamma irradiation on thyme microbial load in order to improve its hygienic quality since thyme is used as a local (Syrian) ethnic cold ready to eat meal. The hygienic quality of non-irradiated (control samples) of thyme meal was estimated by calculating the total viable count (TVC), total coli form (TCC) and moulds and yeast count (MYC) that exhibited rather a high microbiological contamination. A 10 kGy dose reduced the TVC, TCC and MYC from 8.25 to 2.97 log cfu. g^{-1}, from 6.54 to less than 1 log cfu. g^{-1}, and from 4.81 to 2.13 log cfu g^{-1}, respectively. However, a dose of 20 and 30 kGy eliminate all micro-flora (TVC, TCC and MYC) from thyme meal. The D_{10} value for TVC, *Salmonella* spp, *E. coli* and *Aspergillus flavus* was determined as 2.014, 0.625, 0.385, and 0.588 kGy respectively. Our data revealed that irradiation significantly reduced the thyme meals microbial loads which gives the maximum value of these safety parameters.

Keywords: Thyme meal, Gamma irradiation, microbial load, water activity

1. Introduction

Consumers are becoming more literate about the benefits of fresh products containing bioactive compounds [1]. Plant products for human nutrition namely aromatic herbs are the richest source of many compounds including flavonoids, polyphenoles, polysaccharides, terpenoids, alkaloids, quinones, carotenoids, sterols, glucosinolates and other sulphur-containing compounds etc. which provide health benefits due to their wide range of biological properties [2,3,4]. Thus, safety and quality of food products are a major concern for many people worldwide due to related food products borne illness [5,6]. Since the ancient times, herbs have been used in many different ways and culinary herbs have been added to food to enhance flavour and improve their organoleptic properties [7].

Contamination of herbs and spices with micro-organisms is well recognized, attributed in part to growing conditions and environment, sanitation and hygiene practices among harvest workers, and lack of good agricultural practices (GAPs) and good manufacturing practices (GMPs) within some developing countries [8,9]. Therefore, post-harvest and post preparation treatments are essential to minimize microbial spoilage and to reduce the risk of pathogen contamination associated with food products [10]. Various post-harvest and physical, chemical or gaseous post preparation treatments may be applied to maintain fresh-like quality with a high nutritional value, and to meet safety standards of fresh products [11,12].

Irradiation offers a potential benefit to enhance microbiological safety of food, and to reach an accepted nutritional and sensory quality through shelf-life extension [13]. Several reports have demonstrated that gamma irradiation method of herbs and spices is an alternative and effective method for decontamination, quarantine barriers in international trade and improving the shelf-life due to its efficiency and high penetration rate [14,15,16]. It is well identified that macro nutrients are not significantly altered due to irradiation [17]. Currently, over 55 countries in the world have accepted the use of irradiation on food, spices, herbs, vegetables and fruit [18].

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Thyme is an aromatic plant widely used for both nutritional and medical purposes and is native to the Mediterranean region [19]. This herb, added to dishes and foodstuff enhances or improves food flavor and also, because of its antioxidant and antibacterial potential, acts as a preservative agent [20,21].

The production of indigenous foods forms a major part of agro-industries in Syria. The Syrian food industry is traditionally dominated by thyme meal as local (Syrian) ethnic cold ready to eat meal. Indeed a thyme meal is consumed not only in Syria, but also in neighbouring countries. However, they are non-sterile and potential survival or some pathogenic micro-organisms and/or post processing contamination before packaging create microbiological risks, and a considerable limitation of marketing. Therefore, the objective of this study was to investigate the effect of different doses of gamma irradiation on microbial, properties of thyme meal as local (Syrian) ethnic ready to eat meals (foods) aiming to improve its hygienic quality.

2. Materials and Methods

2.1. Thyme meal preparation

A study was conducted on thyme meal in 2016 and 2017 in the radiation technology department, atomic energy commission. Thyme meals were a kind gift from Sedi Hisham (Al-Akkad Company for industry and trade Syria-Damascus countryside). Thyme meal, a local (Syrian) ethnic ready to eat meals (foods) is considered as one of the commercial prepared meal in Syria.

Thyme meal is a traditional Syrian food consisting of several dried ingredients such as sesame, thyme leaves, sumac, coriander, aniseed, fennel, cumin, pistachio, vegetable oil, salt and caraway.

Thyme meals were weighed as in the sampling plan and transferred into polyethylene pouches for irradiation. Each pouch of thyme meal (500 g) was considered as a replicate. The determinations were made in triplicate for each treatment.

2.2. Irradiation treatment

The thyme meals were exposed to gamma irradiation of varying dosages say 10, 20 and 30 kGy, at room temperature, using a gamma source ^{60}CO (ROBO, Russa) with a dose rate of 7.775 kGy h^{-1} at the time of experiments. The absorbed dose was confirmed by alcoholic chlorobenzene dosimeter [16]. The irradiated samples were kept together with the un-irradiated samples as control for 12 months at ambient temperature (18-25 ^{o}C) under relative humidity (RH) of 50-70%.

2.3. Microbiological evaluation

Microbial load was performed on both samples (irradiated and un-irradiated) immediately after irradiation, and after 6 and 12 months of storage. Standard plate count method [22] was used to enumerate the total microbial load in terms of colony forming units (cfu) in control and irradiated samples after 0, 6 and12 months of storage. Total viable count (TVC) was determined on diagnostic plate count agar (PCA) (Oxoid, CM 325, UK). Samples were incubated at temperature 37 ^{o}C for 48 hr. The total coliform count (TCC) was determined on Violet Red Bile Agar (VRBA) (Oxoid, CM 485, UK) at 37 ^{o}C for 48 h. Total mould and yeast (TMY) were enumerated on Dichloran Rose- Bengal Chloramphenicol Agar (DRBC) (Merck, 1.00466, Germany) after incubation at 25 ^{o}C for 5 days. Microbial counts were demonstrated as log 10 cfu. g^{1}.

To determine the survival curves, the thyme meal was artificially inoculated by thoroughly mixing it with a pure culture of TVC, TYM, *Salmonella* spp and *Escherichia coli*. The survival curve was estimated from irradiation doses of 0.2, 0.4, 0.6, 0.8 and 1.0 kGy.

The survival level of *Salmonella* spp was determined by plate counting on Xylose Lysine Desoxycholate Agar (XLD) (Biolife, 402206, Italy) and the survival level *of E. coli* was determined by plate counting on Eosin Methylene Blue Agar (EMBA) (Oxoid, CM 69, UK), after 2 days of incubation at 37 ^{o}C.

2.4. Chemical analysis

Thyme meal samples were homogenized and analyzed in triplicates, to determine moisture (drying for 6 h at 105 ^{o}C) using standard methods [22]. Water activity was determined using the reference solutions [23].

2.5. Statistical analysis

The significance differences among treatments (0, 10, 20 and 30 kGy) at 0, 6 th, and 12th months of storage were determined by analysis of variance (ANOVA) using the SUPERANOVA computer package (Abacus Concepts Inc, Berkeley, CA, USA; 1998). Differences were considered significant at the $p<0.05$ level. D_{10} values were calculated using Cricket graph computer package (40 Valley Stream

Parkway Malvern, PA 19355, 1986/87/88 Cricket Software, Copyright, Version 1.3).

3. Results and discussion

3.1. Microbiological qualities of thyme meal

The hygienic quality of non-irradiated (control samples) of thyme meal as estimated by total viable count (TVC), total coliform count (TCC) and moulds and yeast count (MYC), exhibited rather a high microbiological contamination. The TVC, TCC, and MYC were for the non-irradiated thyme meal 8.25±0.09 log cfu. g^{-1}, 6.54±0.16 log cfu. g^{-1}, and 4.81±0.24 log cfu. g^{-1}, respectively (Table 1).

Non-irradiated samples of thyme meal had high microbial loads and this was attributed to deficiencies in the production protocols, and to lack of standard processing procedures.

In Syria, sesame, thyme leaves, sumac, coriander, aniseed, fennel, cumin, pistachio, vegetable oil, salt and caraway are the major ingredients used in the preparation of thyme meal. These ingredients are sold from local markets to wholesaler who transports the product to city market. In cities, most of the thyme product that is manufactured or processed is unsafe for consumption as it is mistreated in varying degree. Open sun drying of such foods results in contamination by insects, bacteria and moulds. Herbs contamination can be attributed to many factors.

Since Bakobie et al. [6] suggested that spices and herbs such as curry powder, thyme, white pepper, paprika are subjected to a microbial contamination at various stages of preparation.

Coliform bacteria were presented in non-irradiated thyme meal samples. The fecal coliform and *E. coli* detected in the samples were an indication of contamination by fresh fecal matter. This might be due to the inadequate hand washing by vendors and absence of good personal hygiene. It was found that thyme meal samples were not complied with the Syrian community product standard. TVC, TCC, and MYC of used thyme meal were found to be comparatively high, which are not in accordance with the Syrian microbial standards food including thyme meal that include less than 5.0 X 104 cfu g-1 in TVC and negative in TCC, and MYC [24].

The moisture content and water activity (Wa) values of thyme meal were 10.25%, and 0.50 at 24 °C respectively. Such a low Wa provides a longer storage life because the absolute limit for microbial growth is >0.6 [25]. The moisture content of herbs may vary from 6-12% depending on the extent of drying and climatic conditions [26]. Water activity of black pepper, onion powder, cumin seeds and oregano has been reported as 0.40, 0.351, 0.40 and 0.49 respectively [6]. The moisture in seasonal crops including herbs and spices is usually low, which largely protect them against microbial spoilage [27].

Water activity of materials may be the most important factor in controlling microorganisms [28]. A low Wa inhibits the fungal spore germination and mould proliferation [29]. Dried spices and herbs are typically classified as low moisture foods, with Wa within the range of 0.02 to 0.60 [30].

In order to improve the purity and safety of the herb products, observation of basic hygiene during preparation, standardization of some physical characteristics such as moisture are desirable. The preservation of foods by drying is based on the fact that microorganisms and enzymes need water in order to be active [26,31,32]. The interventions in production process are mostly based on reduction or elimination by means of heat treatment [33].

Moulds and yeast were presented in thyme meal samples. In this study, the most abundant fungi isolated from thyme meal were species of *Aspergillus* and *Pencillium*. These species can growth at Wa values below 0.8. Materials with an aw < 0.8 are usually colonized by Aspergillus and Pencillium [34]. Fungal growth and mycotoxin production are the results of a complex interaction between diverse biotic and abiotic factors. Among these factors, Wa and temperature have been described as the most important pre- and post-harvest environmental factors [35].

Mould growth on damp-building materials depends mostly on Wa value [36]. Recently, recalls and food-borne illnesses associated with low-moisture foods (with Wa <0.6) such as spices and herbs have drawn great attention from public, industry, and research communities [37].

3.2. Effect of gamma irradiation on microbiological safety of thyme meal

Data of microbial composition of thyme meals treated with 10, 20, and 30 kGy of gamma irradiation compared with control samples are shown in Table 1. Irradiating the thyme meal reduced the microbial load significantly. A dose of 10 kGy reduced TVC, TCC and MYC from 8.25 to 2.97 log cfu. g^{-1}, from 6.54 to less than 1 log cfu. g^{-1},

and from 4.81 to 2.13 log cfu. g^{-1}, respectively. However, a dose of 20 and 30 kGy eliminate all micro-flora (TVC, TCC and MYC) from thyme meal (Table 1). This result was in good agreement with previous findings on microbial quality of licorice root powders [38], licorice root products [39], aniseed [40] and chamomile powder [26] after irradiation at 10 kGy. The 4 log reduction of total mesophilic bacteria count in the wild thyme was achieved by 5 kGy [20]. Some previous studies showed that gamma irradiation dose of 5 kGy was sufficient to reduce up to an acceptable level the microbiological contamination of herbs, spices or other dried food ingredients [41,42,43].

Table 1. Effect of gamma irradiation on total viable count (TVC) (log10 cfu g^{-1}), total coliform count (TCC) (log_{10} cfu g^{-1}), moulds and yeast count (MYC) (log_{10} cfu g^{-1}) of thyme meal stored at room temperature (18 – 25 °C).

Storage period /(Months)	**0**	**6**	**12**	**P-level**
Treatments	**Total bacterial count (log_{10} cfu g^{-1})**			
Control	**8.25±0.09^{aB}**	**9.35±0.04^{aA}**	**<10.6^{bC}**	**0.0001**
10 KGY	**2.97±0.03^{bB}**	**3.13±0.07^{bB}**	**3.72±0.27^{aA}**	**0.0028**
20 KGY	**>1^{c}**	**>1^{c}**	**>1^{c}**	
30 KGY	**>1^{c}**	**>1^{c}**	**>1^{c}**	
P-level	**0.0001**	**0.0001**	**0.0001**	
	Fungal count (log_{10} spores g^{-1})			
Control	**4.81±0.24^{aC}**	**4.81±0.24^{aB}**	**5.80±0.11^{aA}**	**0.0011**
10 KGY	**2.13±0.22^{bB}**	**2.42±0.18^{bB}**	**2.76±0.03^{bA}**	**0.0099**
20 KGY	**>1^{c}**	**>1^{c}**	**>1^{c}**	
30 KGY	**>1c**	**>1^{c}**	**>1^{c}**	
P-level	**0.0001**	**0.0001**	**0.0001**	
	Total coliform(log_{10} cfu g^{-1})			
Control	**6.54±0.16^{aC}**	**7.60±0.14^{aB}**	**7.99±0.08^{aA}**	**0.0001**
10 KGY	**>1^{bB}**	**>1^{bB}**	**2.36±0.12^{bA}**	**0.0001**
20 KGY	**>1^{b}**	**>1^{b}**	**>1^{c}**	
30 KGY	**>1^{b}**	**>1^{b}**	**>1^{c}**	
P-level	**0.0001**	**0.0001**	**0.0001**	

^{abc} Means values in the same column not sharing a superscript are significantly different.
^{ABC} Means values in the same row not sharing a superscript are significantly different.
NS: not significant.

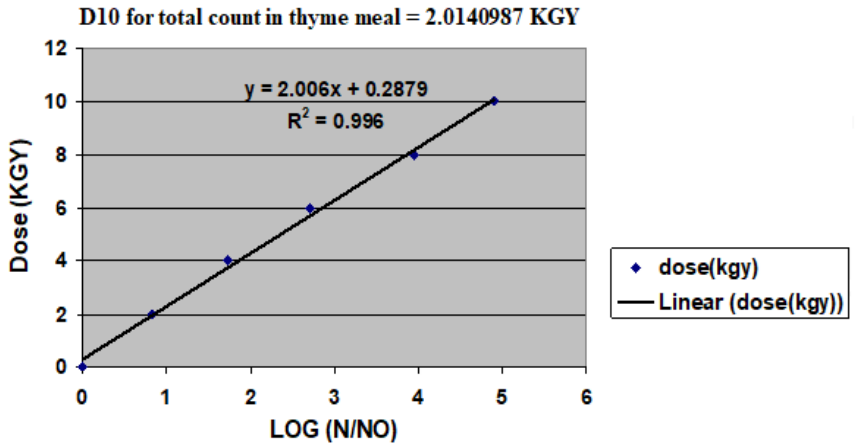

Figure 1. Behavier of total count in occulated in thyme meal samples, subjected to gamma radiation with doses of 0; 10; 20 and 30 KGY (Three replicates).

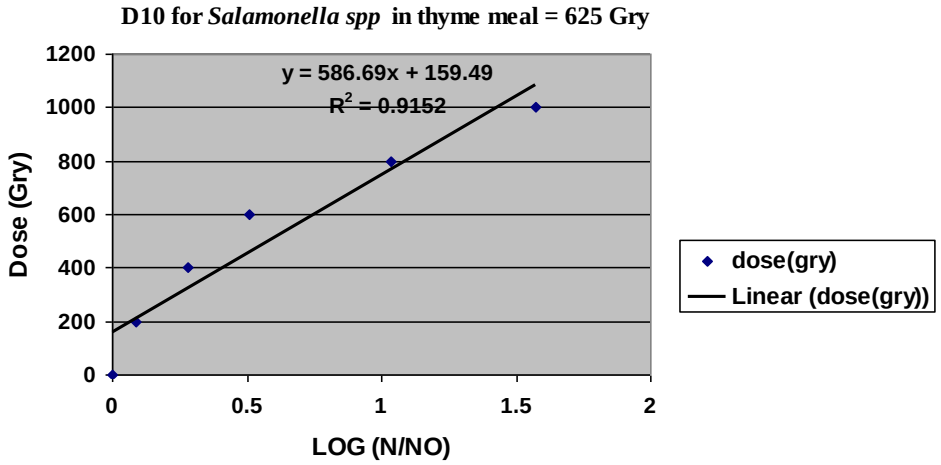

Figure 2. Behavier of *Salamonella spp.* in occulated in thyme meal samples, subjected to gamma radiation with doses of 0; 10; 20 and 30 KGY (Three replicates).

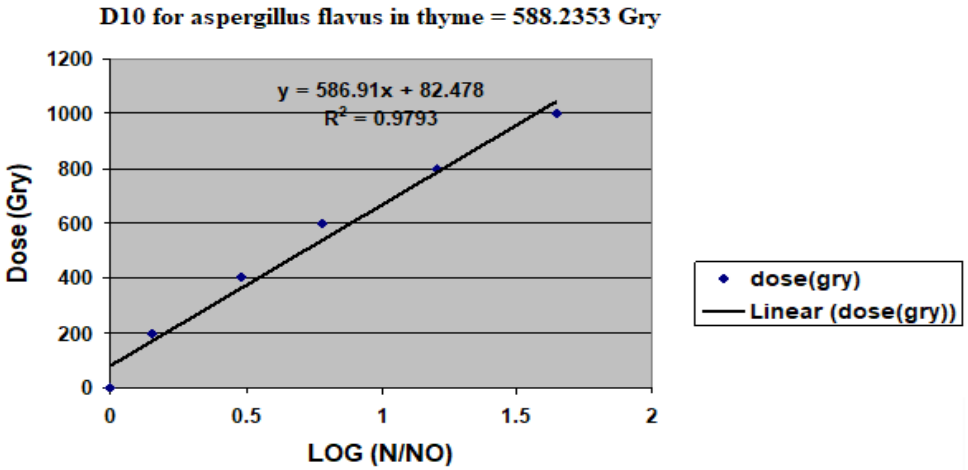

Figure 3. Behavier of *Aspergillus flavus.* in occulated in thyme meal samples, subjected to gamma radiation with doses of 0; 10; 20 and 30 KGY (Three replicates).

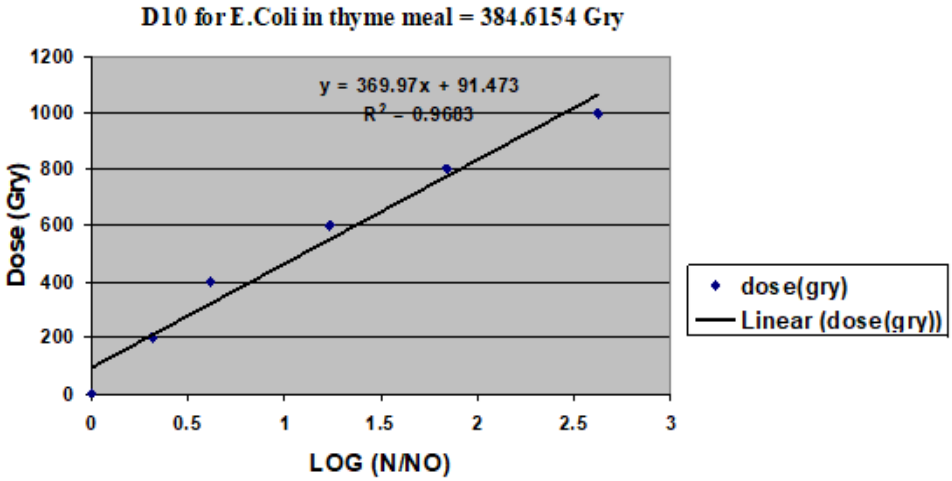

Figure 4. Behavier of *E.Coli.* in occulated in thyme meal samples, subjected to gamma radiation with doses of 0; 10; 20 and 30 KGY (Three replicates).

Efficacy of gamma irradiation on minimizing microbial load of thyme meals may be associated with its ability of penetration deep into tissues and destroying microorganisms harbored in surface or inside host tissues, thus preventing or minimizing the contamination by inhibiting the growth of these microbes [9].

The effect of ionizing irradiation on microorganisms may occur directly or indirectly through the enhanced generation of reactive oxygen species (ROS). ROS can be extremely harmful to organisms [44]. The most important target of ionizing radiation in microorganisms is the DNA molecule that can cause a cell to lose its ability to survive or reproduce as explained by Moreira et al. [45].

The D_{10} value (decimal reduction dose) is the radiation dose required to inactivate 90% of viable bacterial population or reduce the population by a factor of 10. Estimation of D_{10} values may be incorporated into risk assessments for designing processes for reduction of microbial populations in food [46].

The D_{10} value was calculated using the survival curve for microorganisms exposed to gamma irradiation. The D_{10} value of TVC, *Salmonella* spp, *E. coli* and *Aspergillus flavus* was determined as 2.014, 0.625, 0.385, and 0.588 kGy respectively (Figures, 1, 2, 3 and 4).

These results are in agreement with a previous study, which indicated that, the dose needed to reduce the microbial load of ground liquorice roots and aniseed by 1 log cycle (D_{10}) was about 2 kGy [38,39,40]. Apart of the above mentioned study, there has been no other work on the field of the effect of gamma irradiation on the microbial load of commercially thyme meal, but similar observations on other kinds of foods were reported by several researchers in different countries. The radiation doses required to reduce the microorganisms load by one log scale (D_{10}) in Sheesh Tawoq were 435 and 385 Gy for the *Salmonella* and *E. coli* respectively [23]. D_{10} value in the range of 0.40 – 0.46 kGy for Salmonella and 0.24 – 0.25 kGy for *E coli* spp in different meat systems has been reported [47].

3.3. Effect of storage time on microbiological safety of thyme meal

During storage aging, it was noted that the number of contaminant colonies increased with storage period for samples irradiated at 10 kGy of gamma irradiation, similar to non-irradiated controls because of the re-growth of the surviving microbial population. Samples treated with 20 kGy and 30 kGy of gamma irradiation remained completely free of TVC, TCC, and MYC thorough the storage period (Table 1). At the beginning of the storage period non-irradiated thyme meal had TVC, TCC, and MYC greater than 8 log cfu. g^{-1}, 6 log cfu. g^{-1}, and 4 log cfu. g^{-1}. The TVC, TCC, and MYC in the same samples reached the value greater than 10 log10 cfu. g^{-1}, 7 log10 cfu. g^{-1}, and 5 log cfu. g^{-1} after 12 months of storage (Table 1). The high microbial load of thyme meal and that increased throughout storage may be attributed to the high concentration of moisture (10.21%,). Heavy TVC, TCC, and MYC growth was visible after 12 months of storage. Therefore, ambient temperature storage is not suitable for shelf-life extension of thyme meal. We explain these results by the fact that the raw materials of thyme meal (sesame, thyme leaves, sumac, coriander, aniseed, fennel, cumin, pistachio, vegetable oil, salt and caraway) have a high number of contents and the storage conditions (at ambient temperature) are suitable to support the rapid growth of such contaminants. Our results are in agreement with result reported on other herbs and prepared meal products, those results indicated that, during storage aging, the number of contaminant colonies increased with storage period for samples irradiated at dose up to 10 kGy of gamma irradiation, similar to non-irradiated controls of Sheesh Tawoq; as prepared meal [23], Chicken Kabab; as prepared meal [48], Chicken sausage; as prepared meal [47], Kubba; as prepared meal [49], chamomile powder [26], and sesame seeds [16].

4. Conclusion

In recent years, numerous ecological and environmentally friendly methods have been extensively studied for removal of various microorganisms pollutants from food products. Among them irradiation treatments, have been widely used for food decontamination.

Irradiation at doses of 10 was effective in decreasing microbial loads (TVC, TCC and MYC) of thymes meal. While, a dose of 20 and 30 kGy eliminate all micro-flora (TVC, TCC and MYC) from thyme meal. Irradiation in this study clearly demonstrated that gamma irradiation is a safe and successful method to improve the microbiological safety.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

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