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Improvement of Microbiological Quality of Hen Egg Powder Using Gamma Irradiation

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Abstract

Eggs and their products such as desserts, confectioneries, bakery mixes, mayonnaise and many convenience foods have been implicated in food-borne disease outbreaks due to microorganism contamination. The effect of gamma irradiation on the presence of microorganisms in egg powder was investigated. Egg powder samples were exposed to several doses of irradiation: 0, 5, 10 and 15 kGy and stored for up to 12 months at ambient temperature (25 ^{o}C). Results indicated that the total viable count (TVC) (5.56 $\log_{10}$ cfu g^{-1}), total coliform counts (TCC) (6.46 $\log_{10}$ cfu g^{-1}) and mold and yeast counts (MYC) (9.12 $\log_{10}$ cfu g) in un-irradiated (control) samples of egg powder were higher than the maximum limits (4.88, 2.00 and 1.70 $\log_{10}$ cfu g^{-1}, respectively). Application of the higher doses (10 and 15 kGy) decreased the TVC, TCC and MYC of the egg powder samples to less than 1 $\log_{10}$ cfu g^{-1} and the counts remained almost constant during storage for 12 months. D_{10} values for *Escherichia coli* and *Salmonella* typhimurium were 0.714 and 0.278 kGy, respectively. Gamma irradiation treatment could be chosen on the basis of preliminary microbiological tests including TVC, TCC and MYC and help improve the hygienic quality by killing and reducing the microorganisms that might be present inside of egg powder to meet national and international standards.

Keywords: Egg powder; Gamma irradiation; Total viable count; Total coliform count; Mold and yeast count

1 Introduction

Egg is one of the most versatile and near perfect foods in nature, and its essential components form a balanced diet (Akpinar-Bayizit, Ozcan, Yilmaz-Ersan, & Gurbuz, 2010; Ndife, Udobi, & Amaechi, 2010).
Microbial contamination of eggs is a well-established phenomenon and has an important economic implication to the poultry industry (Farag et al., 2012; Wong & Kitts, 2002). Eggs become infected through a process of either transmission, or with moist faces contaminated with *Salmonella*. Following traversing of the eggshell, the associated membrane of the egg becomes permeable to *Salmonella* and other pathogens (Dadashi, Kiani, Rahimi, & Mousavi, 2017; Holt et al., 2011; Jaffer & Nazal, 2013; Nemeth et al., 2011).
Safety of the internal compartments of eggs, in the new alternative poultry production systems, could be microbiologically altered (Holt et al., 2011; Jaffer & Nazal, 2013). Consequently, researchers emphasized the need to rapidly remove any microbial contamination in order to reduce the risk of microbial penetration into the egg contents (Al-Bachir & Zeinou, 2006; Farag et al., 2012; Tan, Kanyarat, & Easa, 2012). Recently,

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the use of shelled egg in food production, as a raw material, has been reduced with the technological developments around the world food industry, and egg products such as frozen egg, and pasteurized liquid egg products have gained popularity (Koc et al., 2011; Ndife et al., 2010). Industrially, dehydrated eggs have many advantages over fresh ones, like inhibition of microorganism development, easier handing and a significant extension in their shelf life (de Jesus et al., 2013; Kumaravel et al., 2011). Drying is a technique used for preserving liquid food, by converting it to a powder form. The whole egg powder obtained by the processes employed, including all methods of drying, was subjected to different studies (Asghar & Abbas, 2012; Schuck et al., 2009).

With growing concerns about food safety, the use of irradiation has been well accepted as one of the best methods for the production of safe meat and poultry (Al-Bachir, 2010; Al-Bachir & Zeinou, 2006). Food irradiation is a non-thermal method, or used as an additional food safety tool, that serves as a compliment to other food safety technologies (Kim et al., 2016). Irradiation of egg and egg products has been used experimentally, as an alternative to heat pasteurization to eliminate *Salmonella*, which is a naturally occurring pathogen in eggs that causes a serious infection (Al-Bachir & Zeinou, 2006; Farag et al., 2012; Kim et al., 2016). Most research on microbial inactivation has tried to determine the proper irradiation dosage to pasteurize egg products. Since 2000, the food and drug administration (FDA), approved the use of up to 3 kGy ionizing radiation dose to reduce the level of *Salmonella* in eggs (Shahin, Swailam, & Abou Zeid, 2006).

The quality evaluation of food products is critical to improve the processing conditions for getting better quality products. Therefore, the aim of the present study was to use gamma irradiation to enhance safety of egg powders, and to analyze the microbial load of an egg powder after irradiation.

2 Materials and Methods

Fresh good quality eggs were obtained from Sidnaia poultry farms, in Damascus Syria. The eggs were candled to confirm their freshness and were cleaned by dusting, washing to remove dirt and other undesirable materials, to avoid any contamination and allowed to dry. They were carefully de-shelled and whole egg liquid obtained in a graduated cylinder. Whole egg liquid was mixed in a blender (WARING commercial blender model 32BL80 made in U.S.A.) for 1-2 min, liquid egg was spread thinly (0.5 – 1.0 mm thickness) on a tray, and oven dried at 60 ^{o}C for 48 hours in a laboratory oven (MEMMERT model 600) to constant mass and allowed to cool. Egg flakes were scooped, milled and sieved with 60 mm mash before being weighed. The egg powders were packed into different plastic films for further investigation.

2.1 Irradiation treatment

Egg powder were irradiated with doses of 0, 5, 10 and 15 kGy, at room temperature, using a gamma source ^{60}CO (ROBO, Russia) with a dose rate of 7.775 kGy h^{-1}. The absorbed dose was monitored by alcoholic chlorobenzene dosimeter (Al-Bachir, 2010). The irradiated and control samples of egg powders were stored for 12 months at ambient temperature (18-25 ^{o}C) under relative humidity (RH) of 50-70%.

2.2 Microbiological evaluation

Standard plate count method was employed to enumerate the microbial load in terms of colony forming units (cfu g^{-1}) in control and irradiated samples after 0, 6 and12 months of storage. Three replicates from each treatment were used, and 10 g of powdered egg samples was homogenized with serial dilutions were prepared according to standard methods (AOAC, 2010). Total viable count (TVC) was determined on diagnostic plate count agar (PCA) (Oxoid, CM 325, UK). Samples were incubated at 30 ^{o}C for 48 hr. Total coliform count (TCC) was determined on a Violet Red Bile Agar (VRBA) (Oxoid, CM 485, UK) at 37 ^{o}C for 48 h. Total mold and yeast (TMY) was done on Dichloran Rose-Bengal Chloramphenicol Agar (DRBC) (Merck, 1.00466, Germany) after incubation at 25 ^{o}C for 5 days. The colony count was reported as colony

forming units per gram of egg powder samples (cfu g^{-1}). Microbial counts were transformed to $\log_{10}$ cfu g^{-1}. To determine the survival curves before irradiation, eggs were artificially inoculated by thoroughly mixing them with a suspension of *Salmonella*. The suspension was prepared by mixing a culture colony of *Salmonella* with pure peptone water media. The *Salmonella* count in the prepared suspension was 10^7 per ml. Before inoculation, eggs were sterilized using gamma irradiation (25 kGy). The ratio of inoculation was 1 ml suspension to 9 ml eggs. The survival curve was estimated from irradiation doses of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 kGy. The survival level of *Salmonella* was determined by plate counting on Xylose Lysine Desoxycholate Agar (XLD) after 2 days of incubation at 37 ^{o}C. Similarly, 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. D_{10} value was calculated using the Cricketgraph (Cricket Software, Maluern, PA, USA) computer package.

2.3 Water activity determination

Water activity was determined using reference solutions (Al-Bachir, 2010).To determine the range capacity and calibration sensitivity of the measurement, the water activity of twelve saturated salt solutions was measured at 20 ^{o}C. Saturation equilibrium of solutions was checked after storage for 2 hr at 25 ^{o}C prior to measurement.

2.4 Statistical analysis

Four treatment doses (0, 5, 10 and 15 kGy), and three storage periods (0, 6 and 12 months) were distributed in a completely randomized design with three replicates for each treatment. Data were subjected to the analysis of variance test (ANOVA) using the SUPERANOVA computer package (Abacus Concepts Inc, Berkeley, CA, USA; 1998). The p value of less than 0.05 was considered statistically significant.

3 Results and Discussions

3.1 Microbiological qualities of egg powder

The extent of contamination by microorganisms in egg powder products was determined. As shown in (Table 1), the mean total viable count (TVC), total coliform counts (TCC) and mold and yeast count (MYC) for the control sample of egg powder were 5.56, 2.62 and 2.33 $\log_{10}$ cfu g^{-1}, respectively. Coliforms are defined as rod shaped gram-negative non-spore forming bacteria. They are a commonly used indicator of sanitary quality of foods. During storage at room temperature, the microorganisms in egg powder products increased gradually reaching, at the end of the storage period (12 months), 9.12, 3.04 and 4.03 $\log_{10}$ cfu g^{-1} for TVC, TCC and MYC respectively, indicating a high contamination percentage of these products. Microbiological population of used egg powder was found to be comparatively high, which was not in accordance with the national and international standards for egg powder that include less than 7.5 X 10^4 cfu g^{-1} in TVC, less than 100 cfu g^{-1} in TCC, less than 50 cfu g^{-1} in MYC (CODEX, 2007; SASMO, 2007). Although MYC contaminated egg powder products were relatively lower than TVC, their counts were also above the safety limits (Table 1). Our previous studies also indicated that the microbial contamination level was above the international and national limits in commercially available dried powder food products found in local markets (Al-Bachir, 2007; Al-Bachir & Al-Adawi, 2015; Al-Bachir, 2017). TVC is indicative of the populations of contaminated microorganisms, and act as an index of hygienic quality. On farms where eggs are produced, the sources of bacterial contaminants have been shown to be surrounding environment, as well as the chickens (Shahin et al., 2006). Microorganisms on the surface of the shell are able to pass through the pores of the shell to contaminate the interior of the egg (Foley & Lynne, 2008; Gantois et al., 2009; Jaffer & Nazal, 2013).

The results of the current research indicated that, *Salmonella* spp and *E. coli* were detected in fresh egg (Table 2). In the case of *Salmonella*

spp and *E. coli* in samples of fresh egg did not meet local and international standards of zero tolerance for food for human nutrition including eggs, absent in *Salmonella* spp, and negative in *Staphylococcus aureus* (CODEX, 2007; SASMO, 2007). The presence of *Salmonella* and *E. coli* in fresh egg samples, as well as in egg powder, may be the result of contamination from the environment and personal or from the raw materials used for preparation. *E. coli* in 10 g egg powder was detected, while *Salmonella* spp. were absent in 25 g egg powder, as demanded in our legislation (Table 2).

Much attention has been given to the role chicken eggs play in the transmission of bacteria, such as *Salmonella* to human populations (Shahin et al., 2006). The TCC and *E. coli* detected in egg powder samples was an indication of contamination by fresh fecal matter. High TCC are usually associated with significant levels of enteric pathogens (Adu-Gyamfi, Torgby-Tetteh, & Appiah, 2012). This, according to FDA (2011), can cause cholera, bloody diarrhea, and kidney failure in people with weak immune systems. Shell eggs and egg-containing products are the most significant sources of *Salmonella* (Min, Nam, Jo, & Ahn, 2012). *Salmonella Enteritidis* and *Salmonella Typhimurium* are the most commonly isolated serotypes in human cases of Salmonellosis, and contaminated egg is a very important source of infection with *S. Enteritidis* for humans (Rakonjac et al., 2014).

3.2 Effect of gamma irradiation on microbiological qualities of egg powder

Application of 10 and 15 kGy doses was enough to decrease the TVC, TCC and MYC in the egg powder to the safety level (to less than 1 $\log_{10}$ cfu g^{-1}), and the counts remained almost constant during storage for 12 months as clearly observed in Table 1. The TVC was found to decrease with a 5 kGy dosage, while TCC and MYC values of irradiated egg powder samples was found to be absent in irradiated samples with the same dose (at 5 kGy). In the present study, the mode of gamma irradiation processing of the egg powder products with the doses of 10 and 15 kGy, were chosen on the basis of preliminary microbiological tests. Since both doses (10 kGy and 15 kGy) completely eliminated microorganisms from the egg powder products. This observation underscores the need for Good Manufacturing Practices (GMP) in production protocols to ensure products have low contamination, and acceptable hygienic quality, and would be in line with the national and international criteria for decontamination of dry foods (SASMO, 2010). This is to be expected, since irradiation is one of the few processes that eliminates disease-causing microorganisms from foods and guarantees high hygienic quality (Adu-Gyamfi et al., 2012). The results corroborate the findings of Aquino, Lui, and Correa (2017), who observed that for the complete elimination of microorganisms in egg powder, it was necessary to use doses around 10 kGy.

Egg disinfection has two purposes. One is to reduce the overall abundance of TVC and MYC that may affect egg respiration and survival. The other is to reduce or eliminate pathogens that may affect egg and fry survival, and compromise the health certification of a hatchery (Barnes, Bergmann, Stephenson, Gabel, & Cordes, 2005). Results of this study indicated that the current practices of gamma irradiation treatment achieve the first and second objectives. This is important for situations in which hatcheries receive eggs from wild sources, or from other hatcheries and processes.

Our results are consistent with literature data, since similar results were obtained in a study performed on dried products similar to egg powder. A previous study showed that gamma irradiation at a dose range of 10–20 kGy was sufficient to eliminate or reduce to an acceptable level, the microbiological contamination of licorice root powders (Al-Bachir & Al-Adawi, 2015), chamomile powder (Al-Bachir, 2017) and aniseed (Al-Bachir, 2007).

Farag et al. (2012) and Adhitia, Octaviani, Rissyelly, Basah, and Mun'im (2017) mentioned that, ionizing irradiation inactivates microorganisms directly by lethal damage of microbial DNA, therefore obstructing bacterial division, and indirectly by free radicals generated during water radiolysis that disintegrate microbial cell membranes.

Table 1: Effect of gamma irradiation and storage period on total viable count (TVC), total coliform count (TCC) and total mold and yeast count (TMY) of egg powder ($\log_{10}$ cfu g^{-1}).

Treatments	Control	5 kGy	10 kGy	15 kGy	P-level
Storage period (Months)		Total viable count (TVC) ($\log_{10}$ cfu g^{-1})			
0	0.05 ± 5.56^{aC}	0.05 ± 3.40^{bC}	>1	>1	0.0001
6	0.18 ± 6.46^{aB}	0.08 ± 3.83^{bB}	>1	>1	0.0001
12	0.29 ± 9.12^{aA}	0.06 ± 4.14^{bA}	>1	>1	0.0001
P-level	0.0001	0.0001			
		Total coliform count (TCC) ($\log_{10}$ cfu g^{-1})			
0	0.08 ± 2.62^{aC}	>1	>1	>1	0.0001
6	0.04 ± 2.84^{aB}	>1	>1	>1	0.0001
12	0.1 ± 3.040^{bA}	>1	>1	>1	0.0001
P-level	0.0001				
		Total mold and yeast count (TMY) ($\log_{10}$ cfu g^{-1})			
0	0.03 ± 2.33^{aC}	>1	>1	>1	0.0001
6	0.21 ± 3.49^{aA}	>1	>1	>1	0.0001
12	0.19 ± 4.03^{aB}	>1	>1	>1	0.0001
P-level	0.0015				

^{abc} Mean values in the same column not sharing a superscript are significantly different.
^{ABC} Mean values in the same row not sharing a superscript are significantly different.
NS: not significant.
* Significant at $p<0.05$.
** Significant at $p<0.01$.

Table 2: Effect of gamma irradiation on Escherichia coli and *Salmonella typhimurium* contaminating egg powder

Treatments	Fresh egg	Egg powder	Egg powder 5 kGy	Egg powder 10 kGy	Egg powder 15 kGy
Escherichia coli	D	D	ND	ND	ND
Salmonella typhimurium	D	ND	ND	ND	ND

D: Detected ND: not detected

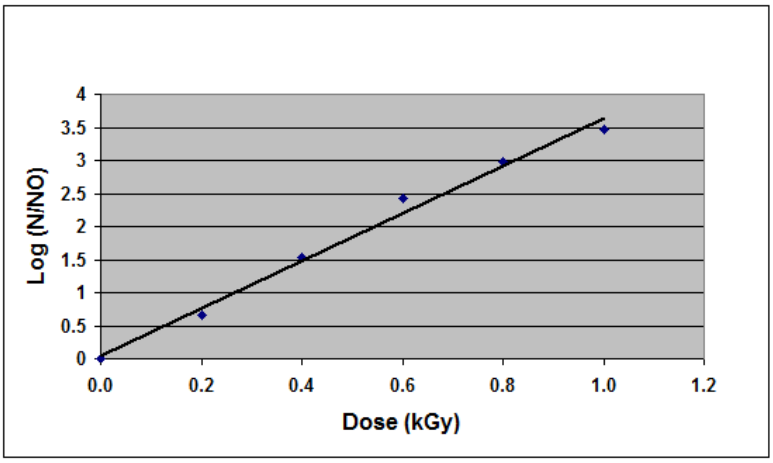

Figure 1: Behavior of *E. coli* inoculated egg powder samples subjected to gamma irradiation at 0, 0.2, 0.6, 0.8, 1.0 and 1.2 kGy (three replicates). (y=3.5929x+0.0488) (R^2=0.9885).

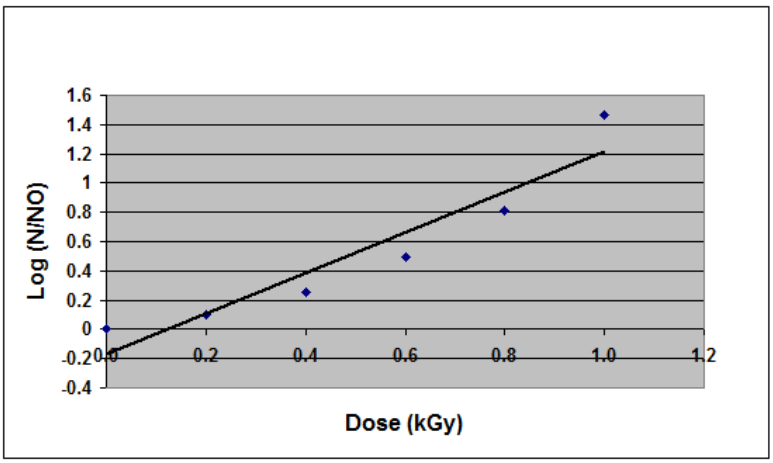

Figure 2: Behavior of *Salmonella* spp inoculated egg powder samples subjected to gamma irradiation at 0, 0.2, 0.6, 0.8, 1.0 and 1.2 kGy (three replicates). (y=1.3901x-0.1738), (R^2=0.8973).

The values of decimal reduction dose (D_{10} value) for *Salmonella* of the powdered egg is 0.714 kGy (Figure 1), while the D_{10} value for *E. coli.* of the powdered egg is 0.278 kGy (Figure 2). Results of the present study are in agreement with those obtained by Froehlich, Gombossy de Melo Franco, Destro, and Landgraf (2015) which indicated that irradiated powdered egg inoculated with *Salmonella* had D_{10} values which varied from 0.76 to 0.86 kGy. Kim et al. (2016) have reported that the D_{10} value of electron beam irradiated egg powder was 0.26 kGy for both *Salmonella typhimurium* and *E. coli.* D_{10} values of bacteria in food are affected by some factors including water activity. The relatively high D_{10} values for both *Salmonella* and *E. coli* in the present study may be due to the lower water content of the egg powder, since the water activity for used egg powder is 0.50 or 0.56% at 24 ^{o}C. Such a low water activity provides longer storage life, because the absolute limit for microbial growth is higher than 0.6 (Kumar, Gautam, Powar, & Sharma, 2010). Although, *Salmonella* spp cannot actively multiply in water below 0.92,they can survive for long periods in eggs (Froehlich et al., 2015). In preserving foods by drying, one seeks to lower the moisture content to a point where the activities of food spoilage and food-poisoning microorganisms are inhibited. Dried or Low Moisture Foods (LMF) are those that generally do not contain more than 25% moisture, and have a water activity (aw) between 0.00 and 0.60. Intermediate Moisture Foods (IMF) have a_w values of 0.60 to 0.85 (with moisture contents of 15 to 50%). They can be eaten without rehydration, but their shelf-life lasts for a relatively long period of time without refrigeration and they are considered microbiologically safe (Syamaladevi et al., 2016).

4 Conclusion

The results of this study demonstrated that the microbiological quality of an egg powder such as total viable counts (TVC), total coliform counts (TC) and mold and yeast counts (MYC) are significantly affected by gamma irradiation. An irradiation dose level of 10 kGy is a promising treatment for decontamination of dried hen egg powder products. The treatment is sufficient to eliminate or reduce TVC, TC and MYC and to maintain products of hygienic quality within safe levels as recommended by national and international food and health organizations either directly after irradiation or during storage.

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