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Full Length Research Paper

Effect of irradiation on inoculated *Salmonella parathyphi* B and the colour of freeze-dried egg yolk

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The effect of irradiation on the colour intensity of freeze-dried egg yolk samples (FDEY), from poultry birds given feed containing different concentrations of annatto extract was investigated. Concentrations of 1, 4, 7 and 10 % of annatto extracts were used in the poultry feed formulation and these were compared with feed containing no additive as well as one containing a commercial feed colourant, 'carophyll yellow'. The FDEY samples coded R_1, R_2, R_3, R_4, C and C_A respectively were inoculated with *Salmonella parathyphi B* and irradiated at doses of 0, 3 and 5 kGy with γ radiation from a ^{60}Co source. Microbial counts and colour intensity were determined after irradiation. Results of the microbiological analysis showed that both 3 and 5 kGy doses were adequate in practically eliminating *Salmonella parathyphi B* from the samples. However, it was observed from the colour analysis that irradiation at both 3 and 5 kGy doses caused significant ($p < 0.0001$) deterioration of the colour of the freeze-dried egg yolk samples.

Key words: Irradiation, *Salmonella parathyphi B,* colour intensity, freeze-dried egg yolk.

INTRODUCTION

Egg is an excellent source of vitamins, minerals and high quality protein and almost devoid of trans fat (Zeisel, 2000). It is one of the few natural sources of some specific nutrients and plays an important role in health maintenance. The binding and colour-imparting properties as well as other functional properties such as flavour-enhancing and the ability to serve as an emulsifier make egg important in food product development (Applegate, 2000).

Egg yolk, which serves as the food source in the egg for the developing embryo, contains all the fat soluble vitamins, (A, D, E and K). It is one of the few foods naturally containing vitamin D and is a rich source of saturated and unsaturated fatty acids as well as lecithin which serves as an emulsifier. The bright yellow colour of egg yolk is due to the presence of the carotenoids lutein and zeaxanthin. The colour of egg yolk is important in the poultry industry since it is commonly believed to be indicative of the health status of birds (William, 1992). As a result, colour concentrates or feed supplements rich in carotenoids are used to improve the colour of the egg yolk. Seeds of the plant *Bixa orellana,* which have a high content of an orange-red pigment (annatto, E160) made up of bixin and norbixin have traditionally been used for colouring in the food industry (Ingram and Francis, 1969). A recent study has also explored its use in enhancing the colour of egg yolk (Ofosu et al., 2010).

Eggs and other poultry products are frequently contaminated by spoilage bacteria such as *Pseudomonas, Xanthomonas, Proteus* and *Serratia*. Additionally, these products harbour other serious pathogens such as *Salmonella* and *Campylobacter* which are responsible for 15 to 62% of contaminations associations with food

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associated with food poisoning (Smith, 1996; Varma, 2005). Studies have established contamination of eggs by Salmonella Entiritidis and Salmonella Galinarum is facilitated by the transovarian route or penetration through the shell and vitelline membrane. Contamination is further promoted by rapid growth of the pathogens in the iron-rich yolk (Humphrey, 1994; Humphrey and Whitehead, 1993). These pathogens can survive extreme levels of temperature, pressure and water activity thus making their elimination difficult (Prescott et al., 1999).

Salmonella contamination of eggs products is a persistent problem and various procedures such as the application of heat, irradiation and combinations of these have been developed to destroy the pathogens. Studies on radiation sensitivity of *Salmonella* species have estimated a wide range of D_{10} under various conditions (ICMSF, 1996). Low dose irradiation is capable of eliminating pathogenic bacteria from eggs. However, irradiating eggs causes damage to the thick white albumen and yolk membranes depending on the dose (IAEA, 1982). Additional studies have also indicated that although irradiation has minimal effect on colour of most foods, it may influence colour by increasing the activity of the enzyme polyphenoloxidase; accelerate or inhibit carotenoid synthesis and intensify anthocyanin formation in certain types of fruits (IAEA, 1982).

Freeze drying of egg yolk improves the shelf life since it greatly reduces the water content of any contaminating microorganisms and enzymes thereby inhibiting their actions. The process however does not completely eliminate pathogens, thus creating food safety concerns. Consequently, the present study considered probable contamination of freeze-dried egg yolk (FDEY) by *S. parathyphi B*, which has been isolated from a number of Ghanaian ready meals (Adu-Gyamfi and Nketsia-Tabiri, 2007), and the possibility of using irradiation to eliminate the pathogen without affecting the colour. The objectives of this study were to investigate the potential of irradiation to eliminate *S. parathyphi B* from FDEY powder, and study the effect of irradiation on the colour of FDEY obtained from poultry birds given feed containing commercial colourant and different concentrations of annatto extract.

MATERIALS AND METHODS

Source and preparation of samples

Freeze-dried egg yolk (FDEY) samples were obtained from poultry layer birds that were fed on poultry feed containing water-soluble annatto extract at concentrations of 1, 4, 7 and 10%, and these samples were coded R_1, R_2, R_3, R_4 respectively. Two other samples C and C_A represented egg yolk from birds given feed containing no annatto and feed containing carophyll yellow (a commercial dye) respectively. A total of six samples were therefore studied. An isolate of *S. parathyphi B* stored on Xylose Lysine Deoxycholate Agar (Difco, USA) slopes at 3 - 5°C, obtained from the Microbiology Unit of the Biotechnology and Nuclear Agriculture Research Institute was used in inoculation of the samples. The FDEY samples were sieved through a 425 µm mesh, packaged in high density polyethylene bags and stored in a refrigerator (5 ± 1°C) until use.

Microbiological analysis

One gram of each sample was added to 9 ml of peptone water (1% peptone water + 0.5% NaCl) and placed on a mechanical shaker (Junior Orbit Shaker, Lab-Line Instruments, USA) for 15 min. Microbial load determination was carried out using standard decimal dilution and plate count methods (APHA, 1976). Total viable counts and coliforms were estimated on Plate Count Agar (Oxoid, UK) and Violet Red Bile Agar (Merck, Germany) respectively at 36°C/48 h. *Escherichia coli* and *Staphylococcus aureus* were also enumerated on Eosin Methylene Blue Agar (Difco, USA) and Baird Parker Agar (Difco, USA) respectively at 37°C for 48 h. In determining counts of *S. paratyphi* B, 5 g of the sample was first pre-enriched in 45 ml of buffered peptone water for 24 h at 37°C, followed by selective enrichment in Rappaport Vassiliadis Soy broth for 24 h at 41°C. Enumeration was done on Xylose Lysine Deoxycholate Agar (Difco, USA) at 37°C for 48 h. The determinations were undertaken in duplicates and each involved double plating of the samples on the appropriate media.

Sample inoculation, irradiation and recovery of *S. parathyphi* B

20 g of each sample was inoculated with 5 ml of the *S. parathyphi* B inoculum of concentration (6.7×10^{-7} CFU/ml) and sealed in a polyethylene pouch using a heat sealer (Heat Sealer, Desk Type: 300 m/m, Taiwan). The inoculated samples were stored at 3 - 5°C for 24 h to allow isolates adjust and then they were treated with irradiation doses of 0, 3, and 5 kGy. Irradiation was done using a ^{60}Co source (SLL, Hungary) at the Radiation Technology Centre of the Ghana Atomic Energy Commission. The measured dose rate was 1.6 kGy/h and Fricke's dosimetry was used to confirm the absorbed dose. The unirradiated and irradiated samples were analyzed for *S. parathyphi B* survivors. One gram of each sample was added to 9 ml of peptone water and placed on a mechanical shaker for 15 min. Survivors were estimated on xylose lysine deoxycholate agar at 37°C for 48 h using standard methods. The experiments were conducted in duplicates and each involved double plating of the samples on the appropriate media.

Colour analysis

Colour intensity of the FDEY samples was determined using Image J software which measures colour intensity in pixels per inch (ppi). Two grams of the non-inoculated samples irradiated at 0, 3 and 5 kGy were added to 5 ml of acetone in Eppendoff tubes, agitated to mix well, and centrifuged at 3600 rpm for 10 min. The supernatants were spotted onto Whatmann's No.1 filter paper, each of diameter 12.5 cm using a 40 µl pipette. The filter papers were air-dried in sterile desiccator and digital scans of both spotted and blank areas were taken using "hp ScanJet 2400" at 1200 dpi. The scanned images were analyzed using Image J (version 1.39) software over selected area of 0.2″ × 0.2″ taken from several spots. Results obtained were reported in pixels per inch (ppi).

Statistical analysis

The microbial counts (colony forming units, cfu/g) were transformed into logarithms ($\log_{10}$). Two-way analysis of variance was used to determine the effects of irradiation dose and concentrations of annatto extract in poultry feed on colour intensity and extent of

Table 1. Microbiological load of freeze-dried egg yolk samples.

FDEY sample	[A] Microbiological index				
	Total viable count	Coliform count	*E. coli*	*S. aureus*	*S. paratyphi B*
R_1	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
R_2	[B]2.72 ± 0.17	< 1.0	< 1.0	< 1.0	< 1.0
R_3	2.82 ± 0.31	< 1.0	< 1.0	< 1.0	< 1.0
R_4	2.54 ± 0.34	< 1.0	< 1.0	< 1.0	< 1.0
C	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
C_A	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0

[A] $\log_{10}$ cfu/g; [B] mean ± S.D., limit of detection = 1.00.

Table 2. Effect of irradiation on survival of *S. parathyphi B* in freeze-dried egg yolk samples.

FDEY sample	[A] Survivors of *S. parathyphi B*		
	0 kGy	3 kGy	5 kGy
R_1	[B]6.11 ± 0.05	< 1.0	< 1.0
R_2	6.20 ± 0.04	< 1.0	< 1.0
R_3	8.05 ± 0.14	< 1.0	< 1.0
R_4	8.34 ± 0.28	< 1.0	< 1.0
C	7.77 ± 0.11	< 1.0	< 1.0
C_A	8.23 ± 0.07	< 1.0	< 1.0

[A] $\log_{10}$ cfu/g; [B] mean ± sd, limit of detection = 1.00.

colour reduction in freeze-dried egg yolk at $p < 0.05$. Least significant difference test was used for mean separation. Statgraphics Centurion XV (www.statgraphics.com) was used for data analysis.

RESULTS AND DISCUSSION

Effect of irradiation on microbiological quality of FDEY

As shown in Table 1, microbiological analysis of FDEY revealed no coliforms, *E. coli, S. aureus and Salmonella* sp. Total viable counts of samples R_2, R_3, R_4 were fairly low between 2.54 and 2.82 $\log_{10}$ cfu/g but no viable cells were detected in R_1, C and C_A. These observations could possibly be due to the preservative properties of freeze drying which generally reduces the water content of substances thus inhibiting survival of microorganisms. Studies have also shown that annatto extract exhibit antimicrobial action against standard strains of gram-positive bacteria including *Bacillus subtilis*, *S. aureus* and *Streptococcus faecalis* (Irobo et al., 1996). The presence of low counts of viable cells (≤ 2.82 $\log_{10}$ cfu/g) in some of the samples (R_2, R_3 and R_4 respectively) could however be attributed possibly to the capacity of certain microorganisms to withstand the freeze drying due to their growth and physiological state.

Effect of irradiation on *S. parathyphi B* in FDEY

No survivors were detected in all the samples of FDEY upon irradiation with doses of 3 and 5 kGy after inoculation with a suspension of 24 h culture *S. parathyphi* B of concentration 6.7×10^7 cfu/ml (Table 2). However, in the case of the unirradiated samples that served as control, *S. parathyphi* B counts of 6.11 to 8.34 $\log_{10}$ cfu/g were recovered.

Salmonellosis continues to be a major food borne disease despite efforts by the food industry. The prevalence of *Salmonella* in poultry products is a food safety concern (CDC, 2006). Irradiation is the only non-thermal method that can efficiently eliminate food borne pathogens such as *Salmonella*, *E. coli* and *Listeria* inside shell eggs. Results of this study have shown that an irradiation dose of 3 kGy can effectively eliminate *S. parathyphi B* from freeze dried egg yolk. This result is very significant considering the fact that the concentration of inoculum used was high (6.7×10^7 cfu/ml). The results is consistent with reported D_{10} values (dose to reduce population by a factor of 10) of 0.66 to 0.76 kGy for *Salmonella typhimurium* in dried egg yoke ((IAEA, 1982). In other related studies, *Salmonella parathyphi B* was eliminated from inoculated jollof rice meal with an irradiation dose of 3 kGy (Adu-Gyamfi and Nketsia-Tabiri, 2008) whiles Byung *et al.* (2008) recommended the use

Table 3. Effect of irradiation on colour intensity of freeze-dried egg yolk samples.

FDEY samples	Colour intensity ($\times 10^3$ ppi)			
	0 kGy	3 kGy	5 kGy	Mean*
R_4	2.20	0.11	0.90	1.07^a
R_3	2.61	0.55	0.51	1.22^a
R_2	1.51	0.35	0.41	0.76^b
R_1	0.77	0.58	0.40	0.58^b
C_A	1.19	0.43	0.24	0.62^b
C	0.43	0.22	0.24	0.29^c
† Mean	1.45^{α}	0.37^{β}	0.45^{β}	

*Means in a column with different superscripts (a-c) are significantly different from each other ($p<0.0001$). †Means in a row with different superscripts (α-β) are significantly different from each other ($p<0.0001$).

Table 4. Effect of irradiation on percent colour deterioration of freeze-dried egg yolk samples.

FDEY samples	Extent of colour deterioration (%)		
	3 kGy	5 kGy	Mean*
R_4	95.49	61.41	78.45^d
R_3	79.69	81.36	80.52^d
R_2	76.18	72.92	74.55^d
C_A	61.84	75.78	68.81^d
R_1	23.88	51.66	37.77^e
C	47.78	42.22	45.00^e
Mean	64.14	64.23	

*Means in a column different superscripts are significantly different from each other ($p < 0.0001$).

of irradiation dose above 2 kGy for controlling *Salmonella* and other pathogens in egg yolk.

Effect of irradiation on colour of FDEY

Colour intensity of the FDEY samples was significantly affected ($p<0.0001$) by irradiation dose and concentration of annatto extract in feed. Both irradiation doses of 3kGy and 5kGy significantly reduced the colour intensity of all the samples (Table 3). The two irradiation doses, however, did not differ significantly from each other with respect to colour intensity. In terms of extent of colour deterioration or reduction, measured as the ratio of the difference in colour intensity between the non-irradiated and irradiated samples to that of the non-irradiated sample, it was observed (Table 4) that applied dose did not have significant effect ($p>0.05$) but the effect of concentration of annatto extract was significant ($p<0.0001$). There was significant interaction between irradiation dose and annatto extract concentration for both colour intensity ($p<0.0001$) and percent colour reduction ($p<0.05$).

Changes in yellowness of egg yolk may be caused by the breakdown of carotenoids in egg yolk by ionizing radiation. However, Byung *et al.* (2008) have reported that irradiation at 2 kGy did not cause substantial changes in physical, chemical, and functional properties such as colour, protein degradation, protein solubility, and emulsion capacity of frozen liquid egg yolk. Irradiation doses used in this study were higher than 2 kGy and therefore may have caused radiation induced chain scission or fragmentation of the bixin and norbixin structures which are the main natural colourants of annatto. This resulted in decreasing colour intensity of the samples upon irradiation. The colour intensities of samples R_3 and R_4 were highest followed by R_2, R_1 and C_A respectively. The FDEY samples from birds that were fed with feed containing neither annatto nor carophyll had the least colour intensity (Table 3). This shows that addition of a natural colourant such as annatto extract to the feed of poultry layer birds can slightly improve or preserve the colour of their freeze-dried egg yolk when irradiated. Mean separation using least significant difference showed that C_A, R_2, R_3 and R_4 suffered higher extent of colour reduction than R_1 and C, implying that both the annatto extract and the commercial colourant carophyll, when expressed in egg yolk, are susceptible to break down by ionizing radiation of 3 kGy and above intended for microbiological decontamination. Hence the higher the concentration of the extract in the feed, the greater was the percent colour deterioration of the FDEY. It is, however, worth noting that FDEY from birds fed with

higher concentration of annatto extract had higher colour intensity.

Conclusion

Ionizing radiation at doses of 3 and 5kGy were effective in completely eliminating *S. parathyphi B* from inoculated freeze-dried egg yolk. However, these irradiation doses caused significant deterioration to the colour of freeze-dried egg yolk from poultry birds given feed containing annatto extract or the commercial colourant caropyhll yellow.

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