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Effect of electron beam on chemical changes of nutrients in infant formula

Adiam Tesfai, Sarah K. Beamer, Kristen E. Matak, Jacek Jaczynski *

West Virginia University, Division of Animal and Nutritional Sciences, P.O. Box 6108, Morgantown, WV 26506, USA

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ABSTRACT

Infant milk formula has recently been implicated as a transmission vehicle for an emerging foodborne pathogen, *Enterobacter sakazakii*, resulting in high mortality rates. Electron beam (e-beam) efficiently and non-thermally inactivates foodborne pathogens, including *E. sakazakii*, in infant milk formula. However, the effects of e-beam on chemical changes of nutrients in infant formula have not been determined. Therefore, the objective of this study was to fulfill this gap. Dehydrated infant milk formula was processed with e-beam at 0 (control) to 25 kGy. Amino acid, fatty acid, and mineral profiles (AAP, FAP, and MP, respectively), as well as protein degradation and lipid oxidation, were determined. There were no differences ($P > 0.05$) in FAP, AAP, and MP. SDS–PAGE electrophoresis qualitatively detected three major protein bands in all samples up to 25 kGy. Densitometry analysis of SDS–PAGE gels confirmed no size degradation ($P > 0.05$) as a function of increased e-beam dose. Totol-volatile-basic-nitrogen (TVBN) excluded ($P > 0.05$) protein degradation due to microbial activity. There was no increase ($P > 0.05$) in lipid oxidation, as assessed with thiobarbituric-reactive-substances (TBARS), except in samples processed at 25 kGy. Dehydrated formula has low water activity, which likely protected nutrients from e-beam-induced chemical changes. This study demonstrates that proteins, lipids, and minerals in infant milk formula are stable when processed with e-beam up to 25 kGy at low temperature and under anaerobic conditions.

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1. Introduction

Infant milk formula has recently been identified as a transfer vehicle for foodborne disease caused by an emerging foodborne pathogen, *Enterobacter sakazakii* (Iversen & Forsythe, 2004; Osaili et al., 2008). Ionising energy is a well-established non-thermal method aimed at improving microbial safety of a wide range of food products. Ionising radiation, such as electron beam (e-beam), gamma radiation or X-rays, efficiently inactivates foodborne pathogens in food products in a non-thermal manner (Arvanitoyannis & Stratakos, 2010; Black & Jaczynski, 2006, 2007, 2008; Chalise, Hotta, Matak, & Jaczynski, 2007; Farkas, 1998; Hvizdzak, Beamer, Jaczynski, & Matak, 2010; Jaczynski & Park, 2004; James, Jaczynski, & Matak, 2010; Levanduski & Jaczynski, 2008; Matak, Hvizdzak, Beamer, & Jaczynski, 2010; Tesfai, Beamer, Matak, & Jaczynski, 2011a, 2011b). A non-thermal antimicrobial process is particularly applicable to infant milk formula because it could potentially prevent nutrient losses associated with thermal processing. It could also be applied to infant milk formula in its final package to reduce chances of cross-contamination. It has been shown that ionising radiation, including e-beam, inactivates *E. sakazakii* in rehydrated and dehydrated infant milk formula (Hong et al., 2008; Osaili et al., 2008). Lee, Oh, Kim, Yook, and Byun (2006) reported no recoverable *E. sakazakii* in infant milk formula processed with gamma radiation at 5.0 kGy. Lee et al. (2006) also reported that a dose of up to 5.0 kGy had no effect on sensory properties of the dehydrated infant milk formula after rehydration and heating.

Ionising radiation is used on more than 60 food types in more than 40 countries (Arvanitoyannis & Stratakos, 2010; Stefanova, Vasilev, & Spassov, 2010). Food irradiation up to 10 kGy is considered unconditionally safe for human consumption (WHO, 1999). Although macronutrients, such as carbohydrates, proteins, and fats, are generally unaffected by irradiation; lipid oxidation and protein degradation have been demonstrated (WHO, 1994, 1995, 1999). These effects are dose-dependent and are highly variable for different food products and processing conditions.

Because nutritional quality of infant milk formula is of critical importance, this is expectedly one of the most highly regulated and controlled food products. Although microbial inactivation of *E. sakazakii* in infant milk formula with ionising radiation, including e-beam, has been relatively well studied, the chemical changes of nutrients in infant milk formula subjected to ionising radiation have received insufficient attention. Therefore, the overall objective of the present study was to evaluate effects of e-beam on

* Corresponding author. Tel.: +1 (304) 293 1893; fax: +1 (304) 293 2232.
E-mail address: Jacek.Jaczynski@mail.wvu.edu (J. Jaczynski).

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chemical changes of major nutrients in infant milk formula. This study was focussed on determination of amino acid, fatty acid, and mineral profiles, as well as protein degradation (proteolytic degradation, by sodium dodecyl sulphate–polyacrylamide gel electrophoresis coupled with densitometry analysis and microbial degradation by total volatile basic nitrogen) and lipid oxidation (thiobarbituric acid-reactive substances) in powdered infant milk formula subjected to e-beam up to 25 kGy.

2. Materials and methods

2.1. Sample preparation

Dehydrated infant milk formula of a national brand was purchased from a chain grocery store. Ionizing radiation may initiate lipid oxidation in food. Since polyunsaturated fatty acids (PUFA) are highly susceptible to lipid oxidation, the formula selected for the present study was fortified with arachidonic (20:4ω6) and docosahexaenoic (22:6ω3) FAs. Individual samples of approximately 25 g were anaerobically packed (10×15 cm Kapak SealPAK pouches, Kapak Corporation, Minneapolis, MN). Prior to e-beam processing, the infant milk formula was evenly spread in each pouch, resulting in a thickness below 3 mm. This thickness ensured complete penetration of e-beam and even distribution of absorbed dose throughout the sample (Jaczynski & Park, 2003a, 2003b). The samples were stored at −80 °C until shipment. The storage time did not exceed 1 week.

2.2. Electron beam processing

Samples were shipped overnight to the e-beam facility (Sterigenics International, San Diego, CA) in a heavy-duty styrofoam cooler filled with dry ice. At the e-beam facility, the samples were allowed to equilibrate to 4 °C overnight in a refrigerator prior to e-beam processing. The samples at refrigeration temperature (4 °C) were subjected to the following target doses: 0 (control); 5, 10, 15, 20, and 25 kGy of one-sided e-beam with energy fixed at 10 MeV. The target doses were confirmed by film dosimetry (Jaczynski & Park, 2003a, 2003b). Film dosimeters (FWT-60 series radiochromatic dosimeters, Far West Technology, Inc., Goleta, CA) were attached to the bottom of the Kapak pouches prior to e-beam processing. The absorbed doses were determined with a spectrophotometer (Cary 100 UV–Vis, Varian, Inc., Palo Alto, CA) at 605 nm. The absorbed doses were 5.7, 11.6, 18.0, 22.9, and 28.7 kGy, respectively.

Immediately following e-beam processing, samples were frozen, packed with dry ice, and shipped overnight back to the food science laboratory at West Virginia University for analysis. Upon arrival, the e-beam-processed samples were stored at −80 °C prior to analysis. The storage time did not exceed one month.

2.3. Amino acid profile (AAP)

The infant milk formula samples processed with e-beam were analysed for full amino acid profile (AAP) according to the AOAC (1995) method 982.30 E (a,b,c). The e-beam-processed samples were subjected to the following three types of hydrolysis: acid hydrolysis with 3 M HCl at 110 °C for 24 h, performic acid oxidation at 0–5 °C overnight followed by acid hydrolysis (3 M HCl at 110 °C for 24 h), and alkaline hydrolysis with fresh 2.1 M NaOH at 110 °C for 22 h. Following hydrolysis, AAs were quantified using the Beckman Amino Acid Analyser (model 6300, Beckman Coulter, Inc., Fullerton, CA), employing sodium citrate buffers as step gradients with the cation-exchange post-column ninhydrin derivatization method (Chen, Tou, & Jaczynski, 2008; Kassis, Beamer, Matak, Tou, & Jaczynski, 2010; Taskaya, Chen, Beamer, Tou, & Jaczynski, 2009). Although full AAP was determined, taurine, lanthionine, ornithine, and hydroxylysine were either not detected or detected at negligible levels; and therefore, are not reported. However, concentrations of these AAs were included in calculations of the total AAs. The data are reported as mean values (±standard deviation) of at least three replicates. The mean values are expressed as g of AA per 100 g of e-beam-processed sample.

2.4. Total volatile basic nitrogen (TVBN)

Protein degradation due to potential microbial activity was determined by total volatile basic nitrogen (TVBN) assay (Goulas & Kontominas, 2005; Tahergorabi, Beamer, Matak, & Jaczynski, 2013). To determine TVBN, 5 g of a sample processed with e-beam were mixed with 50 ml of distilled deionized water (dd H_2O). One drop of silicone and 2 g of magnesium oxide were added to prevent foaming. The mixture was distilled in a Micro-Kjeldahl unit and the distillate was titrated with 0.05 M HCl. The TVBN values are reported as mean values (±standard deviation) of at least three replicates. The mean values are expressed as mg of nitrogen per 100 g of e-beam-processed sample.

2.5. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) and densitometry analysis

Proteolytic degradation, of protein and/or protein polymerisation, was determined by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) in conjunction with densitometry analysis. SDS–PAGE is considered a qualitative analysis, while densitometry is quantitative. 0.4 g of a sample processed with e-beam was dissolved in 40 ml of dd H_2O. The same protein concentration (between formula samples processed at different e-beam doses) was verified by Lowry assay (Lowry, Rosenbrough, Farr, & Randall, 1951). An aliquot of 10 μl of the dissolved sample was mixed with 10 μl of Laemmli sample buffer (Bio-Rad, Richmond, CA) and heated at 90 °C for 5 min (Jaczynski & Park, 2004). Aliquots of 20 μl per well were used for SDS–PAGE.

Ready-to-use 4–20% gradient gel (Bio-Rad, Richmond, CA) was used for SDS–PAGE. SDS–PAGE was performed under denaturing conditions and constant current at 80 V (Jaczynski & Park, 2004). The Precision Plus Protein™ Kaleidoscope™ protein standards (10–250 kDa) (Bio-Rad, Richmond, CA) were added to the SDS–PAGE, along with the e-beam-processed samples. The electrophoretic protein patterns were stained with EZ-Run protein staining solution (Bio-Rad, Richmond, CA), followed by distaining with dd H_2O.

The SDS–PAGE gel images were captured using a digital camera interfaced with a PC (Fluorchem 8000, Alpha Innotech Corp., San Leandro, CA), using transluminating white light (Alpha Innotech Corp., San Leandro, CA) (Gigliotti, Davenport, Beamer, Tou, & Jaczynski, 2011; Kassis, Gigliotti, Beamer, Tou, & Jaczynski, 2012). The optical density of protein bands from SDS–PAGE images were analysed, using the Fluorchem software (version 1.0, Alpha Innotech Corp., San Leandro, CA). The densitometry data are reported as mean values (±standard deviation) of at least three replicates. The mean values are expressed as integrated pixel density of a protein fraction. A sample representative SDS–PAGE gel image is also reported.

2.6. Fatty acid profile (FAP)

The infant milk formula samples processed with e-beam were analysed for fatty acid profile (FAP). The e-beam-processed samples were subjected to lipid extraction with acid hydrolysis into ether, followed by their methylation to fatty acid methyl esters

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