ELECTRON BEAM PROCESSING FOR EGG PRODUCTS

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

Electron Beam Processing of Egg Products

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This research explores electron beam processing of eggs and its impact on their functionality as an ingredient in processed foods. Electron beam processing (EBP) is recognized as an alternative to traditional sanitizers for the inactivation of *Salmonella typhimurium* and *Escherichia coli* cells in raw foods. While this technology has potential applications in the egg processing industry, information about how EBP affects the foaming, emulsifying, and gel-forming properties of eggs once they are introduced into product formulations is limited. To model these key properties, eggs exposed to 3 kGy of target dose were used to produce standardized recipes of meringue, mayonnaise, and custard. Properties including pH, gel texture parameters, foam density, foam stability, and emulsion viscosity were measured and compared to an unprocessed control group. This study found that electron beam processing has a statistically significant effect on products made with egg albumen; samples in the treatment group displayed increased foam formation speed, foam stability, and gel hardness. EBP did not appear to have an effect on emulsion viscosity or product pH.

DEDICATION

I would like to thank Dr. Suresh Pillai for the opportunity to undertake this challenge, and for his dedication to making sure that I actually do it right. A thank you to Dr. Bailey for letting me use his expensive equipment, and to Hector Leyva for teaching me how it works. I would also like to thank Sohini Bhatia for her insight, edits, and reassuring words.

I would like to dedicate this research first to my mom and dad, for giving me the trust, support, and freedom that allowed me to pursue this research wholeheartedly. Second, to my brother Garret, who is living proof that it is possible to be talented at nearly everything and still be genuinely humble. Lastly, I'd like to dedicate this paper to my friends in the Aggie Speleological Society, for their ability to pull me out of my own thoughts during rough times, and for teaching me that there are few fears that can't be conquered by curiosity. Cave on, guys.

CHAPTER I

INTRODUCTION

Electron beam processing is a non-thermal food processing technology that has the potential to greatly improve food safety and shelf life without affecting the sensory qualities of the treated food. This technology uses ionizing radiation to cause single and double-strand breaks in microbial DNA, rendering them unable to grow and reproduce (Pillai 2016; Miller 2005). EBP treatment of raw produce, like eggs, is of particular interest since it is a non-thermal process that can be used when the product needs to remain uncooked but still requires pasteurization.

Salmonella spp. has been a pathogen of concern for the egg industry for many years, and has cause numerous outbreaks that are costly both to industries and the consumer. Current sanitation methods for raw shell eggs involve treatment with chemical sanitizers, which can fail to completely eliminate pathogenic bacteria from the surface of the egg (Morouj N and others 2016). Low doses of electron beam radiation have proven effective in producing a significant reduction in *Salmonella spp.* counts in multiple types of fresh produce (Tahergorabi and others 2012). Doses of radiation as low as 2 kGy can reduce bacteria such as *Salmonella spp.*, *Listeria*, and *E. coli* to undetectable levels (Wony and others 2003).

While we know the effects of EBP on the microbiology of fresh produce, its impacts on sensory quality, particularly when the food undergoes further processing, have remained largely unexplored. One study of the physicochemical properties of EBP eggs found a reduction in gelling and foaming capacity, but no change in ability to form emulsions after treatment (Wony and others 2003). A study of isolated egg albumen protein found that higher doses of ionizing radiation will reduce the bound water content and cause moisture loss, which could potentially

affect the final water content of the egg product (Jin and others 2017). These studies indicate that raw egg white protein undergoes chemical changes when exposed to ionizing radiation. My focus was on the qualities of whole egg products that have undergone some form of secondary thermal or nonthermal denaturation after EBP. I chose meringue, custard, and mayonnaise because these are ideal foods to demonstrate the foaming, gelling, and emulsification properties of irradiated eggs, respectively. The objective and underlying hypotheses are as follows:

- **Objective 1:** To determine the effects of EBP on pH of raw egg albumen and yolk and determine if any pH changes are still detectable once the eggs have been processed into RTE foods.
- **Hypothesis 1:** It was hypothesized that EBP would cause a pH increase in the raw egg, but that further processing would negate this change.
- **Objective 2:** To determine the effects of EBP on egg's ability to rapidly form stable foams by observing the properties of meringue formulated with EBP eggs.
- **Hypothesis 2**: It was hypothesized that eggs treated with approximately 3kGy of dose would produce egg foam with increased volume and enhanced stability.
- **Objective 3:** To assay the effects of EBP on egg's ability to form oil-water emulsions by observing the properties of mayonnaise formulated with EBP eggs.
- **Hypothesis 3:** It was hypothesized that eggs treated with 3kGy of dose would produce a more viscous emulsion than the control.
- **Objective 4:** to assay the effects of EBP on egg's ability to form strong gels by observing the properties of custard formulated with EBP eggs.
- **Hypothesis 4:** it was hypothesized that eggs treated with 3kGy of dose would produce gels with no detectable change textural qualities.

CHAPTER II

LITERATURE SURVEY

The literature survey for this paper was done systematically. My attempt was to be as far-reaching as possible while staying focused on the main topic. A list of pertinent search terms was compiled and run through the Library of Congress, LISTA, PubMed, Texas A&M Library, and Web of Science Core Collection Databases. Results were evaluated for their relevance to the objectives of this research, as well as relevance to the necessary background required to understand its motivations and results. Literature pertaining to the effects of gamma and UV processing on egg products was excluded. Articles addressing electron-beam processing of non-food substances, live organisms, and pathogens not found in eggs were excluded. Given the volume of existing literature on irradiated food products, papers directly pertaining to eggs or egg derivatives were preferred over those addressing other food systems. This literature review did not attempt to judge the quality of specific methodologies, and instead attempted to review as many sources as possible and explain the differences in methodology in studies with conflicting results.

CHAPTER III

LITERATURE REVIEW

Pathogen Profile of Raw Eggs

The inclusion of raw eggs in a product presents a potential microbiological hazard. Pathogens of concern for raw eggs include *Salmonella Spp., Escherichia coli,* and *Listeria Monocytogenes,* with *Salmonella Spp.* being the primary pathogen of concern (Min and others 2005). Pathogenic *Salmonella Spp.* is a major issue in ready to eat (RTE) foods because it can survive thermal pasteurization in some food processes and go unchecked in instances where thermal treatments are not appropriate, such as in raw produce (Tahergorabi and others 2012). Raw chicken eggs continue to be a major food vehicle for foodborne illness because pathogens can penetrate the shell and contaminate the albumen portion of the egg (Tahergorabi and others 2012).

Need for Alternative Sanitizing Methods

Quaternary ammonium compounds (200ppm) and chlorine-based sanitizers (100ppm available chlorine) are the most common sanitation techniques currently in use for raw shell eggs (Morouj N and others 2016). Chlorine efficacy is dependent on a number of factors including contact time, concentration, and the organic load of the product or wash waters, which can neutralize the sanitizer and decrease its efficiency (Morouj N and others 2016). A combination of 8 minutes of UV irradiation and a 3.5% hydrogen peroxide spray has been found to be especially effective in sanitizing shell eggs, but like other forms of chemical sanitation, it is not effective on pathogens that have penetrated into the inner portion of the egg (Morouj N and others 2016).

Furthermore, chemical sanitizers are not an option when dealing with liquid eggs that have already been removed from the shell. Ionizing radiation remains the only non-thermal process that can deactivate pathogens that have penetrated into the albumen portion of raw shell eggs (Narvaiz and others 1992). A study of the radiation sensitivity of *Salmonella spp.* isolates inoculated into raw eggs revealed that while cells on the interior of raw eggs are more resistant to EBP, a dosage of 1.5 kGy is enough to achieve at least a 4 $\log_{10}$ reduction in *Salmonella spp.* throughout the whole egg.

Ultraviolet Irradiation

UV irradiation is a technique that has been used on both in-shell and liquid eggs as a means of cold pasteurization. Samples of liquid egg treated with UV demonstrate significant reduction in *Salmonella spp.*, but the process only tolerates a product thickness of 3 mm before effectiveness is lost due to low beam energy (Abdanan Mehdizadeh and others 2015). UV is also known to require lengthy exposure times for effective microbial inactivation (Palekar and others 2015). UV irradiation was found to increase foam ability and stability of egg foams (Abdanan Mehdizadeh and others 2015). UV irradiation is non-ionizing, but can produce free radicals that mimic the effects of EBI, thus similar effects on physicochemical properties may be expected.

Electron Beam Technology

Electron beam radiation is a form of ionizing radiation that, rather than requiring a radioactive isotope, is produced from the acceleration of electrons via an electromagnetic pulse. A linear accelerator, or linac, produces radio wave frequencies that are amplified within a vacuum tube called a klystron (Pillai 2016). The produced RF wave accelerates the free electrons

produced by an electron gun component. The resulting electron beam is run through a magnetic scanner that widens the beam into a fan-like shape (Miller 2005). Higher-throughput facilities may have an electron beam on either side of the processing line to ensure a uniform dose without requiring multiple passes through the beam (Pillai 2016).

The nutritional, toxicological, and microbiological wholesomeness of ionizing radiation as a food processing method has been under intense scrutiny from the scientific community for over 60 years, and has thus far been considered a safe and effective means of treating foods (Farkas 2006). Ionizing radiation does not leave behind chemical residues or radiolytic products, and has been approved by the FDA and USDA for use on a wide variety of foods, including a maximum allowable dosage of 3kGy for raw eggs (Pillai 2016). Forms of ionizing radiation that have been approved for use in food processing include electron beam, X-ray, and gamma technology using either a Cobalt-60 or Cesium-137 isotope. Electron beam technology has a comparatively lower penetration depth limit relative to X-ray or gamma technologies; the upper penetration limit for a 10 MeV beam is 3.9 cm, assuming that the product is a high-moisture food (Farkas 2006).

Electron beam technology produces similar effects to the gamma which is already in widespread use in food processing, however with several logistical and processing benefits. Firstly, electron beam processing does not require the storage or disposal of radioactive isotopes, which are costly and dangerous to handle (Turman and others 2002). Second, electron beam technology has a more uniform and controllable dose rate compared to gamma irradiation technology, leading to a product with a more uniform dose (Song and others 2017). Due to the expenses related to acquiring and handling radioactive isotopes, most producers are beginning to favor forms of ionizing radiation produced by an accelerator, particularly electron beam

processing (Miller 2005). More importantly, the throughput of eBeam processing far exceeds that of cobalt-60 based gamma processing.

The effects of ionizing radiation are quantified by the amount of radiation absorbed by the product, called the dose. Tools used to measure dose, called dosimeters, are available in several types, commonly including plastic, radiochromatic dye, and alanine pellets and films. Alanine pellets are currently the most accurate form of dosimeter, giving readings within 2% of the actual delivered dose (Miller 2005).

Mechanics of Microbial Inactivation by EBP

A linear electron accelerator produces a beam of electrons with sufficient energy to eject electrons from atoms and molecules. This ionization results in two types of effects: the breaking of chemical bonds (primary effects), and the production of short-lived radicals from surrounding water molecules that will react with nearby molecules (secondary effects). Accelerated electrons inactivate microorganisms by damaging the nucleic acids within microbial cells, either through direct impact with an accelerated particle or by the production of a reactive radical species that can react with the nucleic acid. Both the primary and secondary effects of EBP will render microorganisms incapable of growth and reproduction, although D_{10} values may differ between individual microorganisms(Farkas 2006). Though electron beam is not capable of targeting DNA specifically, DNA is especially sensitive to ionizing radiation because of its size compared to other cellular components (Miller 2005). Evidence suggests that one kGy of ionizing radiation can produce approximately 20 double-strand breaks in a cell's genome (Asaithamby and Chen 2009).

Microbial Responses to EBP

Many factors can influence a microorganism's response to EBP, including temperature, growth medium, and growth cycle of cells (Miller 2005). Sporulating bacteria and organisms that can survive in low water activity environments can be particularly resistant to ionizing radiation due to the decreased formation and mobility of hydrolytic radicals (Miller 2005). *Salmonella spp.* has proven the ability to develop radioresistance after repetitive irradiation at non-lethal dose, likely due to the development of new outer membrane proteins (Tesfai and others 2011). The D_{10} value of *Salmonella spp.* is known to range from 0.5-0.7 kGy depending on the culture (Miller 2005; Tesfai and others 2011). Research has shown that 1.5 kGy of EBI is sufficient to reduce *Salmonella spp.* levels by approximately 4 log10 in liquid and shell eggs (Serrano and others 1997).

Dosimetry of Shell vs Liquid Eggs

Dose mapping simulations of in-shell eggs revealed that the egg's irregular shape results in a poor dose uniformity ratio that may be cause dosing inconsistency (Kim and others 2011). To overcome this, eggs are removed from their shells and irradiated in a thin layer. The study also stated that electrons only penetrated 0.6 cm into the egg when a low energy (1.35 MeV) beam was used, but most electron beams used for food irradiation run at between 8.5 and 10 MeV, giving them better penetration. To ensure dose uniformity, the eggs used for this experiment had a depth of 0.5cm and were processed with a 10 MeV beam.

pH Changes During EBP

Research indicates that the pH of raw egg yolks treated with 2.5 kGy of EBI experienced significant pH increase that persisted through the first 15 days of storage (Huang and others 1997). However, a more recent study on raw egg white did not indicate a pH change at any dose (Min and others 2005). This research will attempt to verify which of the two studies is accurate, and assay whether any existing pH changes will affect the physical properties of foods containing EBP eggs. A pH change in egg yolks may interfere with functional properties of food proteins and cause a change in how the eggs interact with ingredients such as chemical leavening agents and starches. Thus far no studies have been done to model the effects of e-beam induced pH changes within processed foods.

Foaming, Emulsion, and Gel Quality of EBP Eggs

The functional properties of foam, emulsion, and gel formation will be a primary area of focus in this study. Data on EBI's effects on these characteristics seems to conflict from source to source. One study indicated a decrease in emulsion capacity, foam stability, and gel hardness when raw shell eggs were treated with 3kGy of EBI (Wony and others 2003). However, different research stated that raw egg yolk samples treated with 2.5kGy of EBI "had a significantly higher emulsion capacity than nonprocessed samples" (Huang and others 1997). Yet another study suggests that when applied to spray-dried egg white protein, radiation dose and egg foam stability are directly related (Clark 1992). There is no published data on the direct impacts of these physicochemical changes on the formulation of food products. This research aims to clear up the confusion on EBI's effects on gel texture by modeling the actual effects of irradiated egg yolks on food systems. Possible effects on product quality caused by these physicochemical

properties are poorly understood and may be of significant interest to the food industry looking to incorporate irradiated eggs into RTE foods.

EBP's Effects on Proteins

Proteins are high molecular weight compounds comprised of hundreds to thousands of amino acids. Given their size, it can be expected that some of the proteins in a treated food will be affected during EBP. Walnut protein treated with 5.0kGy of dose were found to be more prone to aggregation and cross-linking, with enhanced thermal stability and no effect on physical stability of the protein (Zhao and others 2017). However, the rigid conformational strain on the amino acid backbone of proteins gives them some degree of resistance to both the primary and secondary effects of ionizing radiation (Miller 2005). Conformational analysis of EBP egg white protein powder showed that 5.4kGy of dose does not interfere with the secondary structure or functional groups of powdered egg white protein (Jin and others 2017). 5 kGy of EBI is enough to produce puncture pores and fragmentation on the surface of proteins, which can lead to partial denaturation and a change in functional properties (Zhao and others 2017). At doses typically required for food sanitation, protein functionality is not affected (Tesfai and others 2014). Small differences in thermal denaturation temperature of egg white protein have been reported following EBP ; raw egg whites treated with 2.5kGy of EBI have been found to denature at temperatures up to 1^{o}C lower than the control (Huang and others 1997). This appears to be a small change, and it is unclear how it may affect the functional properties of raw egg. Pea proteins treated with high doses of EBP experience an increase in emulsification and foaming capacity, but these properties begin to degrade once more than 10kGy of dose is delivered (Wang

and others 2017). This research intends to discover the impact that EBP may have on the functional properties of proteins within raw egg yolk and albumen.

EBP's Effects on Carbohydrates

EBP has little to no effect on the nutritional quality of carbohydrates, but can weaken macromolecule structures such as amylose or cellulose in food systems (Miller 2005). Higher doses of EBP have the capacity to break glycosidic linkages between carbohydrate monomers and crosslink certain polysaccharides to form hydrogels (IAEA 2016). 5.4kGy of EBI was found to cause perforations in corn starch granules that significantly reduced the water holding capacity and viscosity of corn starch gels (Xue and others 2017). Total reducing sugar content in starch or cellulose containing foods tends to increase with dose due to the cleavage of glycosidic bonds (Wei and others 2014). Whole eggs contain only about 1% carbohydrates by weight, thus physicochemical changes related to carbohydrate breakdown from EBP are expected to be negligible.

EBP's Effects on Lipids

High doses of EBI can initiate lipid oxidation and promote rancid odors and flavors in foods, particularly those with a high unsaturated fatty acid content. This effect is much less pronounced at the sub-3kGy doses typically used for cold pasteurization, and can be mitigated by excluding oxygen from the processing environment (Miller 2005). Carcinogenic products caused by high-temperature cooking of lipids, such as aromatic or heterocyclic rings, do not form during EBP (Miller 2005). Lipid oxidation caused by EBI has been effectively mitigated in food

systems by both natural and synthetic antioxidants, offering a solution to any oxidation that may be caused by the process (da Trindade and others ; Nam and others 2007).

EBP's Effects on Vitamins

Vitamins are small molecules that are not typically affected by the primary effects of small to medium doses of EBI. Vitamins with antioxidant capabilities may be consumed by the radical products of EBP's secondary effects (Miller 2005). Ascorbic acid, thiamin, and vitamins A and E are among the most radiation sensitive vitamins (Miller 2005). The chemical changes in vitamin composition caused by EBP do not pose any glaring nutritional concerns, particularly given the regulations on maximum received dose for foods (Kilcast 1994). The losses in vitamin content at doses practical for food processing are known to be comparable to those caused by common food storage or cooking (David and Kilcast 1989).

CHAPTER IV

MATERIALS AND METHODS

Electron Beam Processing

USDA AA grade large eggs purchased from a local grocery store were for both the control and treatment groups in this experiment. The eggs were used within their sell-by date. To produce EBP liquid eggs with a consistent dose of approximately 3.0kGy, fresh in-shell eggs were first cracked and separated into yolk and albumen components by hand. 24 eggs were separated for each of the three replicates. Yolks were homogenized by breaking the yolk with a fork and whisking vigorously until homogenous. 60ml of either egg yolk or white was filled by autopipette into 14x18cm whirl-pak bags. The bags were then heat sealed and labeled with date of preparation. The final product had a thickness of approximately 5.0mm. Air was excluded from the package to prevent inconsistencies in dose caused by bubbles. Samples were prepared and then immediately refrigerated, then e-beam processed once they had reached 10^{o}C. Samples were placed in the refrigerator immediately after processing.

Dose Mapping

The following dose-mapping study was conducted at the National Center for Electron Beam Research at Texas A&M to ensure that uniform dose could be achieved with the specified processing method. Two alanine pellet dosimeters were placed on the underside of each bag, in the positions indicated by the arrows in figure 1**.**

Figure 1. Dosimeter placement.

The dosimeters were placed in both a central and perimeter location to account for any changes in thickness that may occur near the edges of the pouch. The samples were run under a 10MeV electron beam that was mounted above the conveyor belt. Optimal belt speed for the desired dose of 3.0kGy was determined by running a “speed check” sample at 30 fpm and incorporatin the received dose, belt speed, and target dose into the equation in figure 2.

$$Target\ Speed = \frac{recieved\ dose\ x\ belt\ speed}{Target\ dose}$$

Figure 2. Belt Speed Determination Equation

The equation determined that 24fpm is the the optimal belt speed to achieve 3.0kGy of dose. Three samples were processed under the beam at this speed to ensure that a uniform dose was being achieved across all samples, and determine whether the egg yolk and egg albumen samples would require different belt speeds to achieve the target dose. Once it was confirmed that a uniform dose was being achieved across all samples, it was determined that all EBP egg

samples for the course of this experiment were to be run at 24fpm. Received dose was monitored during all experimental replicates by the NCEBR's dosimetrist, using alanine pellet dosimeters placed on select samples. Both the center and the edges of each whirl-pak were monitored, ensuring that each sample received a uniform dose throughout the package.

pH Measurements

A Corning^{TM} 430 pH meter was used to determine the pH of each sample of raw egg yolk or albumen used in each of the textural assays. The pH meter was calibrated with a pH 7 and pH 10 buffer solution before testing was performed. The pH of meringue, custard, and mayonnaise samples was also recorded immediately after their preparation. To test solid samples, a slurry of each food was created by mixing 15 g of the sample into 15ml of deionized water and shaking until homogenous.

Meringue Preparation

Meringue was prepared according to the following formula:

Table 1. Meringue ingredients.

Ingredient	**Amount**
Egg albumen	120g
Tartaric acid	1g
Granulated sugar	25g

All ingredients were placed in the bowl of a stand mixer and whipped with whisk attachment at maximum speed for 2 minutes. The product's physicochemical properties were assayed immediately after preparation.

Foam Stability of Meringue

The stability of meringue's foam structure was assessed in order to give insight into how EBP may affect the shelf life of egg foams. Immediately after preparation, 50ml of meringue were filled into a 50ml graduated cylinder. The sample was allowed to rest for 24 hours at room temperature, after which the volume of liquid that had collected at the bottom of the cylinder was recorded. The experiment was performed in triplicate for both EBP and untreated meringues.

Specific Density of Meringue

Specific density of the meringue samples was calculated to assess the egg's ability to trap air after EBP. 300ml of meringue was placed in weighing dish and weighed. Specific density was calculated using the formula in figure 3.

$$\frac{Weight\ (g)}{Volume\ (ml)} = Specific\ Density\ (g/ml)$$

Figure 3. Specific Density Equation.

Mayonnaise Preparation

Control-group mayonnaise was prepared with the following formula, in accordance with US Code of Federal Regulations standards of identity for mayonnaise:

Table 2. Mayonnaise ingredients.

Ingredient	**Amount**
Egg yolk	40g
Morton salt	1 g
Fresh lemon juice containing no solids	20ml
HEB brand dijon mustard	4g
HEB brand vegetable oil	240 ml

Liquid egg yolk at 10ºC was placed in the bowl of a Hamilton Beach™ 63391 stand mixer. Egg yolk, salt, lemon juice, and mustard were mixed for 15 seconds at the mixer's maximum speed using the whisk attachment. The mixer was then run continuously as the vegetable oil was added slowly over the span of 2.5 minutes. The mixer was stopped, and the sides of the bowl were scraped down with a rubber spatula to incorporate remaining solids. The mixer was then run again for 30 seconds on maximum speed until homogenous. The product was packed into sealed whirl-pak bags and refrigerated at 10ºC until testing. The treatment group samples were prepared using the same protocol, save for the inclusion of EBP eggs in place of untreated eggs.

Viscosity of Mayonnaise

A viscosity assay was performed on the mayonnaise immediately after preparation to determine how EBP may have affected mouthfeel and particle size of the emulsion. A U.S.

Solid™ rotary viscometer was used for this assay. The protocol for testing viscosity of mayonnaise samples is as follows: The viscometer was plugged into the AC adapter and powered up. The viscometer was switched on and the protective cap was removed from the rotor connector. Rotor #4 was screwed into the rotor connector and rotor #4 was selected on the selection screen. On the selection screen, rotor speed was set to 6rpm, output was set to print, and clock was set to "display". 10°C mayonnaise was filled into a 140ml beaker, and the rotor was lowered into the beaker. The "OK" button was pressed to start data collection. Data was recorded in mPa's. The rotor was washed with deionized water between samples. The rotor was raised out of the beaker and cleaned with soap and water, then placed back into its case for storage.

Custard Preparation

The ingredients used to prepare the custard can be found in table 3 below.

Table 3. Custard Ingredients

Ingredient	**Amount**
HEB Regular Cream Cheese	225g
Egg yolk	90g
Egg Albumen	180g
Carnation Sweetened Condensed Milk	415ml
Eagle Brand Evaporated Milk	355ml

10°C cream cheese was beaten on maximum speed in the bowl of a stand mixer with whisk attachment for 60 seconds. The remaining ingredients were then added, and the mixture was

whipped for an additional 60 seconds. The mixer was stopped, and the sides of the bowl were scraped down with a rubber spatula. The mixer was then run again for 60 seconds on maximum speed. The mixture was poured into three ceramic ramekins, which were placed in a metal baking tray. Three ramekins of custard made with control eggs were baked simultaneously with three ramekins of custard made with EBP eggs. The arrangement of the EBP and control ramekins in the oven was rotated for every replicate to account for the possibility of hotspots in the oven. The tray was filled with 2 cm of water to ensure even heating throughout the ramekins. The tray was then placed in a preheated oven at 165^{o}C for 45 minutes. Once baked, the tray was drained of water and covered in aluminum foil, then placed in a refrigerator allowed to cool to 10^{o}C overnight. Samples were held at 10^{o}C until analysis for no longer than 24 hours.

Texture Analysis of Custard

A texture analysis was conducted on custard samples to assess gel quality and predict how they may behave when chewed. Gel characteristics of 3.75cm square of custard with a height of approximately 2.8cm were determined with a TA.XT Plus texture analyzer with a 7.5cm plunger. The sample diameter was made to be smaller than the plunger diameter, to mimic the action of the food being pressed to the top of the mouth by the tongue (Pons and Fiszman 1996). The simplified texture profile analysis macro on the TA.XT Plus allowed for simultaneous recording of values for hardness, adhesiveness, resilience, cohesiveness, springiness, gumminess, and chewiness. Samples were each tested at refrigeration temperature, 10^{o}C. Three samples were taken from each replicate, using samples cooked near the front, center, or back of the oven during cooking.

Statistical Analysis

The experiments in this study were replicated three times, with each performed on a separate day to control for experimental variability. A batch of egg albumen and a batch of egg yolk were e-beam processed for each replicate; the batches were then used to perform the pH measurements, meringue preparation, foam density assay, foam stability assay, mayonnaise preparation, mayonnaise viscosity, custard preparation, and custard texture analysis protocols. Each replicate also included a set of parallel control samples using control eggs. Control and treatment groups for each replicate were tested within the same day.

The data produced by these experiments was analyzed through single factor ANOVA with a significance factor set at 0.05. ANOVA was used to detect statistically significant differences between treatment and control groups. Dosimetry data was separated into readings for egg yolk and egg white, then the received dose was reported as the average received dose ± standard deviation within the sample.

CHAPTER V

RESULTS AND DISCUSSION

pH Measurements

There was no statistically significant difference between the pH of untreated liquid eggs and eggs treated with 3kGy of EBP. Furthermore, no pH difference was detected between treatment and control groups for any of the further processed egg products. This seems to indicate that the 3kGy dose did not produce a pH change detectable by the power of this assay, and that pH was not a factor in any of the observed differences between control and treatment groups.

Table 4. Raw Yolk pH

Replicate	**Control**	**EBP**
1	6.51	6.41
2	6.37	6.39
3	6.46	6.33
Average	6.446667	6.376667
Standard Deviation	0.070946	0.041633

Table 5. Raw Albumen pH

Replicate	**Control**	**EBP**
1	8.22	8.98
2	8.7	8.38
3	8.22	8.53
Average	8.38	8.63
Standard Deviation	0.226274	0.254951

Table 6. Meringue pH

Replicate	**Control**	**EBP**
1	6.84	6.62
2	7	7.09
3	6.77	6.74
Average	6.87	6.816667
Standard Deviation	0.096264	0.199388

Table 7. Mayonnaise pH

Replicate	**Control**	**EBP**
1	3.66	3.55
2	3.64	3.54
3	3.76	3.62
Average	3.66	3.55
Standard Deviation	0.052493	0.03559

Table 8. Custard pH

Replicate	**Control**	**EBP**
1	6.32	6.31
2	6.03	6.23
3	6.33	6.31
Average	6.226667	6.283333
Standard Deviation	0.139124	0.037712

Meringue Density

A statistically significant difference was observed between the density of meringue foam made with EBP eggs and that made with control eggs. Meringue made from eggs whites treated with 2.89 ± 0.08 kGy of dose showed a statistically significant decrease in their density, indicating that EBP egg albumen has enhanced foam-producing capabilities when compared to

the control. It is likely that EBP caused a change in the surface hydrophobicity of egg albumen proteins, lining up with the research of Wang and others (2017) that observed a change in the surface hydrophobicity of Pea proteins treated with doses up to 10kGy. This change is likely a function of alterations in surface protein conformation, an effect first reported by Clark and others (1992). These findings indicate that EBP could provide a benefit to processors in that it allows egg whites to form strong foams with less processing time.

Table 9. Meringue Density

Replicate	**Control**	**EBP**
1	0.18	0.12
2	0.17	0.12
3	0.17	0.14
Average	0.17	0.12
Standard Deviation	0.01	0.01

Meringue Stability

There was a statistically significant difference between the amount of runoff collected from EBP meringue foam and control meringue foam over a period of 24 hours. Meringue made with EBP treated eggs displayed enhanced stability when compared to the control, indicating that EBP could offer a shelf life benefit to manufacturers of egg-foam products. The underlying cause of this change is also likely related to conformational changes on the surface of egg protein affected by EBP. The increased stability of EBP egg foams provide a clear shelf-life benefit to producers of stable egg foams

Table 10. Meringue Stability

Replicate	**Control**	**EBP**
1	7	5
2	7	5
3	7	6
Average	7	5
Standard Deviation	0	0.47

Mayonnaise Viscosity

No statistically significant difference was recorded between the viscosity of mayonnaise made with EBP egg yolks and mayonnaise made with untreated egg yolks. Any viscosity difference would have indicated a change in egg yolk's ability to properly emulsify polar and nonpolar phases of a food product, but the lack of a detectable difference suggests that egg yolk's emulsifying properties are unaffected by electron beam processing. This data suggests that food processors should be able to benefit from EBP's food safety benefits without having to put effort into product reformulation.

Table 11. Mayonnaise Viscosity

Replicate	**Control**	**EBP**
1	68600	57000
2	33300	30800
3	27600	28300
Average	43166.67	38700
Standard Deviation	18134.01	12980.24

Custard Texture

The study failed to detect a statistically significant difference associated with any of the textural parameters of custard, aside from hardness. Hardness is a measurement of the force required to achieve a pre-determined deformation of a product. The fact that there was a detectable difference between EBP and control custards indicates that there is a definite change in the food's gel-forming properties as a result of electron beam processing, but it is unclear whether it is a large enough change for consumers to detect. Because none of the other textural parameters showed statistically significant differences between EBP and control samples, it is unlikely that the textural change could be considered a major defect or benefit to the technology. It should be noted that texture profile analysis is still an emerging field at the time of publication, and that any data produced by it should be confirmed by sensory analysis studies or other forms of assay.

Table 12. Custard Hardness

Replicate	**Control**	**EBP**
1	1495.602	2166.749
2	1396.924	2005.836
3	1537.769	1825.831
4	1457.877	1533.506
5	1075.23	2108.275
6	1021.325	1706.822
7	1918.69	1293.506
8	1351.318	1602.585
9	1748.579	1555.314
Std. Dev.	269.8132	276.8347

Table 13. Custard Adhesiveness

Replicate	**Control**	**EBP**
1	-339.048	-330.723
2	-303.567	-310.253
3	-312.653	-301.609
4	-328.773	-340.154
5	-331.181	-378.68
6	-262.532	-284.421
7	-304.168	-298.67
8	-259.416	-317.253
9	-290.287	-290.843
Std. Dev.	-339.048	-330.723

Table 14. Custard Resilience

Replicate	**Control**	**EBP**
1	10.59	12.846
2	8.58	9.767
3	11.424	10.464
4	12.938	9.33
5	10.143	16.701
6	9.139	17.956
7	26.537	19.778
8	26.206	24.908
9	20.329	23.137
Std. Dev.	6.852686	5.504812

Table 15. Custard Cohesion

Replicate	**Control**	**EBP**
1	0.339	0.392
2	0.307	0.33
3	0.345	0.331
4	0.392	0.271
5	0.366	0.455
6	0.285	0.461
7	0.608	0.511
8	0.6	0.587
9	0.508	0.565
Std. Dev.	0.116556	0.104044

Table 16. Custard Springiness

Replicate	**Control**	**EBP**
1	54.023	62.376
2	50.99	50.99
3	55.446	55.446
4	66.584	42.079
5	68.317	75.99
6	42.327	75.99
7	82.426	78.96
8	85.891	77.475
9	27600	28300
Std. Dev.	43166.67	38700

Table 17. Custard Gumminess

Replicate	**Control**	**EBP**
1	498.138	848.824
2	428.223	660.957
3	530.151	604.499
4	571.102	415.845
5	393.267	959.244
6	290.93	787.692
7	1166.331	660.349
8	810.69	941.192
9	27600	28300
Std. Dev.	43166.67	38700

Table 18. Custard Chewiness

Replicate	**Control**	**EBP**
1	285.41	529.46
2	218.35	337.02
3	293.95	335.17
4	380.26	174.98
5	268.67	728.93
6	123.14	598.57
7	961.36	521.42
8	696.31	729.19
9	609.59	695.79
Std. Dev.	256.53	185.94

CHAPTER VI

CONCLUSIONS AND RECCOMENDATIONS FOR FURTHER RESEARCH

Effects on Egg Albumen

It is theorized that the underlying cause of observed changes in the foaming or gel-forming properties of eggs resulting from electron beam processing can be attributed to conformational changes in the albumen proteins that give egg white its structural properties. The results from Wang and others (2017) indicates that eBeam can cause disruptions in the secondary structure of proteins and cause notable conformational changes on their surface. It appears that the dosage of 3 kGy is sufficient to produce this effect in egg albumen proteins. A change in surface conformation likely exposed a variety of functional amino acid groups that were not previously on the exterior of the protein. This could include amphiphilic amino acid groups which would alter the surface hydrophobicity of the protein, affecting how it interacts with water and lipids within the food matrix.

A change in surface hydrophobicity would account for changes in the way egg white forms gels during cooking and forms foams during agitation. The exposure of amphiphilic amino acid groups would account for the increase in gel hardness, as it would allow for lipid and water phases of the gel to bind more effectively. It may also account for the change in the rate at which air could diffuse out of the egg foam. Albumen proteins are responsible for egg's ability to disperse air throughout a liquid and keep it bound within a foam, therefore a change in the surface properties of albumen protein would have a direct effect on foam's formation and stability (Lorient and Dickinson 1995).

If the theory that EBP is causing conformational changes in albumen protein holds, it is likely that there would be a small but detectable change between control and EBP groups. It is recommended that further studies perform a more precise analysis of the pH change during EBP, with more controls and replicates to ensure that no difference exists. The detection of a pH change during EBP would likely confirm the theory that EBP disrupts the surface conformation of albumen proteins, even at doses as low as 3kGy.

The role of EBP egg albumen in baked goods including cakes and pastries may also be an interesting area of study, given the enhanced foaming abilities of EBP eggs.

Effects on Egg Yolk

While it is unclear whether the apparent change in gel hardness caused by EBP was a result of the yolk or albumen proteins within the gel matrix, table 11 indicates that the emulsion forming properties of egg yolk were unaffected by the treatment. From this it is inferred that the amphiphilic proteins responsible for egg yolk's emulsion forming capabilities are unaffected by the 3kGy dose, suggesting some level of resistance to e-beam's effects. It may be possible that another compound within egg yolk is responsible for this resistance, or that the amphiphilic protein structures are simply more resilient than those in egg albumen. Regardless, food industry professionals should be able to use EBP treated egg yolk without requiring product reformulation.

Organoleptic Concerns

It is crucial that future research investigate the organoleptic properties of foods made with EBP treated eggs. This study made no attempt to assay consumer acceptance, palatability,

or the concentration of relevant flavor compounds. An off-odor coming from treatment-group products was detected during the course of the experiment, suggesting that EBP may have caused the release of unpalatable aromatic and volatile compounds that could be a detriment to consumer enjoyment of EBP foods. An analysis of sulfur and aromatic compound concentration and a simple discriminative sensory analysis are both recommended for future inquiries.

Recommendations to Industry

It is recommended that egg processors carefully optimize the dose to obtain the desired changes yet avoid negative effects in terms of aromatic and volatile compound production within egg-based foods. Improvements in processing efficiency will also be required to make this a cost-effective process. Should sensory analysis prove EBP products to be palatable to consumers, methods of streamlining the treatment of large quantities of liquid egg should be investigated. . Flow-through systems would be needed (similar to UV processing of fruit juices) to make this technology suitable for industrial adoption.

Summary Statement

Electron beam processing remains a promising alternative to traditional methods of combating pathogenic organisms in raw eggs. EBP has the potential to provide a considerable safety benefit to consumers and demonstrate potential processing and logistical benefits for the food industry. Although further research will be required to ensure that EBP egg products are acceptable to consumers, this technology appears to have many potential applications within the egg processing sector, as well as the potential to be applied to other protein-based foods.

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APPENDIX

DOSIMETRY REPORTS

Process Control No Mason Rackley 3.5.18

Set opened on 3/5/2018 11:52:27 AM Calibration Name Probe Low 1-9-18

Indiv. Mass ☐ Batch No. B0311

Indiv. Irr. Temp ☐ Insert PL0083

Min. Dose [kGy] 2.83

Max. Dose [kGy] 2.94

Sample No	Dose [kGy]	Alanine	Marker	Alanine / Marker	Freq. [GHz]	Instr. Temp.	Measured on	User	Comments
Sp Ck Yolk	2.89	37.293	95.139	0.398	9.77030	36.4	3/5/2018 11:55:32 AM	admin	
Sp Ck Whites	2.94	37.424	94.072	0.404	9.77044	36.3	3/5/2018 12:00:48 PM	admin	
Yolks 1	2.85	36.727	95.235	0.392	9.77013	36.2	3/5/2018 12:07:29 PM	admin	
Whites 1	2.83	36.596	95.505	0.389	9.77044	36.2	3/5/2018 12:07:51 PM	admin	
Whites 2	2.87	36.085	92.915	0.395	9.77017	36.3	3/5/2018 12:08:20 PM	admin	

Monday, March 05, 2018 1 / 1

Process Control No Mason Rackley 3.12.18

Set opened on 3/12/2018 9:01:44 AM

Indiv. Mass ☐

Indiv. Irr. Temp ☐

Min. Dose [kGy] 2.79

Max. Dose [kGy] 2.98

Calibration Name Probe Low 1-9-18

Batch No. B0311

Insert PL0083

Sample No	Dose [kGy]	Alanine	Marker	Alanine / Marker	Freq. [GHz]	Instr. Temp.	Measured on	User	Comments
SpCk	2.83	38,690	100,735	0.389	9.77066	37.0	3/12/2018 9:22:24 AM	admin	
Yolks - 1	2.94	39,841	99,315	0.405	9.77021	37.7	3/12/2018 9:23:22 AM	admin	
Yolks - 2	2.98	40,659	99,809	0.411	9.77067	37.8	3/12/2018 9:25:10 AM	admin	
Yolks - 3	2.83	38,464	99,744	0.389	9.77057	37.8	3/12/2018 9:25:35 AM	admin	
Whites - 1	2.86	38,654	98,862	0.393	9.77045	38.6	3/12/2018 9:41:29 AM	admin	
Whites - 2	2.79	37,809	98,895	0.384	9.77037	38.6	3/12/2018 9:41:54 AM	admin	
Whites - 3	2.97	39,797	97,734	0.409	9.77053	38.7	3/12/2018 9:42:24 AM	admin	

Process Control No Mason Rackley 3.14.18

Set opened on 3/14/2018 10:36:15 AM
Indiv. Mass ☐
Indiv. Irr. Temp ☐
Min. Dose [kGy] 2.70
Max. Dose [kGy] 3.01

Calibration Name Probe Low 1-9-18
Batch No. B0311
Insert PL0083

Sample No	Dose [kGy]	Alanine	Marker	Alanine / Marker	Freq. [GHz]	Instr. Temp.	Measured on	User	Comments
SpCk Whites	2.94	41,337	102,292	0.404	9.77065	39.6	3/14/2018 10:38:44 AM	admin	
SpCk Yolks	2.70	37,897	102,156	0.371	9.77072	39.5	3/14/2018 10:39:27 AM	admin	
Whites 1	3.01	42,190	101,640	0.415	9.77052	39.8	3/14/2018 10:46:28 AM	admin	
Whites 2	2.81	39,107	101,100	0.386	9.77068	39.8	3/14/2018 10:47:19 AM	admin	
Yolks 1	2.97	41,901	102,331	0.409	9.77073	39.7	3/14/2018 10:47:51 AM	admin	
Yolks 2	2.80	39,559	102,567	0.385	9.77074	39.7	3/14/2018 10:48:32 AM	admin	