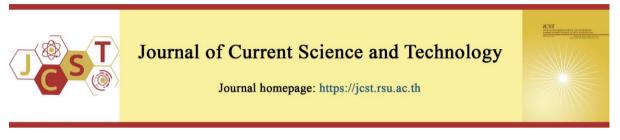
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Effect of Electron Beam Irradiation Doses on Quality and Shelf Life Extension of Non-Allergenic Ready-to-Eat Plant-Based Meat and Egg

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Abstract

This research evaluated the effectiveness of electron beam irradiation (EBI) on a shelf-life extension of ready-toeat plant-based burger patties and hard-boiled eggs. The products were irradiated at different doses of the electron beam (2, 3.5, and 5 kGy) and microbiological assays (total aerobic bacteria, yeast and mold, *Clostridium perfringens*), quality parameters (color, firmness, a_w, moisture content, TBA content), and sensory quality of the products were determined. The results indicated that EBI at 2-5 kGy did not affect on physical, chemical qualities and sensory of the products. However, microorganisms were found in the products irradiated at 2 kGy. Therefore, irradiation of products at the dose of 3.5 kGy was chosen for evaluating their nutritional qualities and shelf-life. The data confirmed that electron beam at 3.5 kGy effectively preserved nutritional value, GABA, and phenolic contents, including ACE inhibitory of plant-based meat and egg. The shelf-life of plant-based hard-boiled egg and plant-based burger patties at room temperature was 217 days or 7.25 months, according to an accelerated shelf-life study at 40° and 50 °C.

Keywords: allergen-free plant-based food; vegan food from rice; electron beam irradiation; non allergen meat; non allergen egg

1. Introduction

There is an increasing need to move toward more plant-based meat and egg to safeguard global food security due to the growing effects of climate change and environmental degradation on food production and dangers related to public health, pandemics, and geopolitical developments. It has been reported that plant-based meat uses 47-99% less land, and 72-99% fewer greenhouse gas emissions than meat. It also promotes human health and animal welfare. Thailand's bio-circular-greeneconomic model (BCG) places a strong emphasis on agriculture as a way to sustainably utilize natural resources. Therefore, the concept of ready-to-eat and easy-to-keep plant-based meat and egg production was innovated. The main raw materials used in these products are food waste and food loss or by-products of Thai rice processing from community enterprises. Diets based on plants emphasize foods that are mostly derived from plants. These diets contain low-fat content and no cholesterol, according to numerous research. They contain dietary fiber and nutrients, which can lower the risk of NCDs (non-communicable diseases) such as obesity, diabetes, heart disease, vascular disease, and cancer (Li, 2014; Lin et al., 2019). Because plant-based foods are nutrient-dense, their market is always expanding. According to Franck (2019), the global market for plant-based meat will cost 27.9 billion US dollars in 2025, growing 15% annually, while the global market for plant-based egg products will cost 1.72 billion US dollars in

2027, growing 6.2% annually, and reaching 140 billion US dollars in 2029.

Protein is the most important nutrient of plant-based food. Almost all plant proteins can be used as the building blocks for meat and egg substitutes. However, this depends on several criteria, including the raw material's accessibility, affordability, and technological readiness. The most used proteins for meat substitutes are soybean, pea, and wheat protein (Choudhury et al., 2020). However, when employed in products, soy protein has sensory drawbacks related to beany, grassy, and bitter flavors (Chamba et al., 2014). Furthermore, wheat protein contains gluten, which can lead to celiac disease or wheat allergies for some customers, and soybeans are the most often genetically modified plants, which may not be acceptable to consumers concerned about the food's naturalness. The development of allergen-free food ingredients has been the subject of numerous attempts, such as rice malt (Puangwerakul, 2006), bio-cellulose from rice kombucha (Puangwerakul, 2018), red rice malt (angkak) (Puangwerakul, & Soithongsuk, 2019), nutrition yeast from Thai rice wine (Puangwerakul, 2019) and rice protein hydrolysate (Puangwerakul, & Soithongthongsuk, 2017). The following is an analysis of the crucial composition of these elements from prior study (Puangwerakul, & Soithongsuk, 2021; Puangwerakul, & Soithongsuk, 2022); B1, B2, B3, B5, B6, GABA, fiber, antioxidants, and enzymes that aid in protein and starch digestion were all present in rice malt; Mevinolin and monacolin K, which have numerous advantages including antiinflammatory, anti-bacterial, and antioxidant activities, were found in red rice malt; Branch-chain amino acids (BCAAs) which serve as brain and muscle builders and ACE inhibitory peptides which help to lower blood pressure were found in rice protein hydrolysate; Rice protein isolate produced the chicken and egg flavoring compounds 2methyl-3-furyl-disulfide, 2-methyl-3-furanthiol, and 2,6-dimethyl-pyrazine; beta-glucan, iron, zinc, and vitamin B12 were all present in dried yeast, while fiber and cancer-inhibiting DSL were present in bio-cellulose. Plant-based meat and eggs are not only one of the main trends for "Foods Today" and "Future Foods" but also present consumer health benefits.

To extend the shelf life of food products, cold sterilization using ionizing radiation is a good alternative for maintaining the original quality of the products as much as possible (Paula et al., 2019). Irradiation is a method that uses high-energy

photons or charged particles to kill microorganisms. Foods exposed to radiation of less than 10 kGy were found to be non-toxic by the FAO, WHO, and International Atomic Energy Agency (IAEA) (Amiri et al., 2019; Chen, et al., 2016, E-BEAM Services, 2014). The use of irradiation technology is prevalent in the food industry. Over 200 irradiated foods have been approved in 50 countries (Li et al., 2019; Cutrubinis et al., 2007). Ionizing radiation by electron beam irradiation (EBI) is promising sterilization that destroys microorganisms, and it is characterized by high dependability, cheap cost, quick process cycle times, and environmental friendliness. It is quite safe, and the E beam sterilized products have a verifiable assurance of sterility, are not radioactive, and do not leave behind any sterilant residues. (E-BEAM Services, 2014). EBI can prevent microbial growth in foods by directly or indirectly harming the microbe's physiological metabolism and chemical processes carried out by the microbes, which can result in injury or death. The energy transfers the microorganisms receive due to EBI causes the denaturation of enzymes and membrane proteins and the breakdown of chemical and molecular bonds by disrupting DNA structure. The result is aging and death since the cells cannot carry out their regular physiological and metabolic functions and lose the ability to replicate and divide their chromosomes. As a result of EBI's ionization of water molecules and generation of unstable free radicals, other cellular metabolic pathways are damaged and intracellular oxidation is promoted, resulting in cellular damage and cell death.

The usefulness of EBI in reducing microbes in raw meat and meat products is widely documented. It can increase an item's shelf life and ensure its general safety. The United States Department of Agriculture has approved using 4.5 and 7.0 kGy irradiation in the microbial decontamination of frozen and refrigerated animal foods. Recent research has demonstrated that EBI significantly raises the total quality of meat and meat products (Lung et al., 2015). However, plantbased foods have different nutrition from real animal-based food and there is little information on the effect of electron beam irradiation on ready-toeat plant-based foods. Therefore, this research aimed to study how low-dose electron beam irradiation affected the quality and shelf-life extension of innovative ready-to-eat plant-based hard-boiled eggs and burger patties.

2. Objective

This research aimed to study how lowdose electron beam irradiation affected the quality and shelf-life extension of innovative ready-to-eat plant-based hard-boiled eggs and burger patties.

3. Materials and methods

3.1 Preparation of plant-based hard-boiled egg and plant-based burger patties

All ingredients for the vegan "meat" and "eggs" were weighed out as follows:

Plant-based hard-boiled egg: 80g/one egg contains 35% rice protein hydrolysate, 34% rice malt powder, 17% PEN EGG; commercial vegan egg powder (Thai FDA13-2-03144-6-0002), 5% dried bio-cellulose powder, 4% rice protein isolate, 3% dried yeast, 1.2% angkak red rice malt powder, and 0.2% salt. For white egg, white egg ingredient was mixed with water in ratio of 1:3. For egg yolk, white egg ingredient was mixed with mashed pumpkin in ratio of 1:1 (w/w). White egg and egg yolk were poured into egg-shaped silicone mold and steam for 15 minutes, then packed in MITSUBISHI DIAMIRON M film and seal under vacuum condition by Thermoform Machine (ULMA TFS500, Spain).

Plant-based burger patties: 50g/ one serving size contains 12.5% fresh bio-cellulose from kombucha, 7.5% rice protein hydrolysate, 7.5% rice malt, 4.5% angkak red rice malt, 2% red bean, 1.5% cockle mushroom, 1% commercial emulsifier, 0.5% rice bran oil, and 0.75% dried yeast. The ingredients were combined, then vegan burger sauce was added in a 20:1 mixing ratio. After the paste had been formed into a burger mold press, (diameter 7 centimeters, thickness 2 centimeters), each piece of burger patty weighed 50 g and steam for 15 minutes then packed in MITSUBISHI DIAMIRON M film and sealed under vacuum condition by Thermoform Machine (ULMA TFS500, Spain).

Samples were packed in MITSUBHI DIAMIRON M film by the Thermoform Machine ULMA TFS 500 (Spain) with following parameters, vacuum switch point: 20 mbar; sealing temperature: 145°C; sealing time: 1.8 seconds; sealing pressure: 4 bar.

All of the components for the plant-based hard-boiled egg and the plant-based burger patties were kindly provided by the Nong Sarai Farmers Group's cooperative business and Incubation Research and Incubation of Entrepreneur Center, Faculty of Food Technology, Rangsit University.

3.2 Effect of electron beam radiation dose on product quality

Electron beam irradiation was performed at the Thailand Institute of Nuclear Technology (Public Organization), Technopolis, Klong 5, Thailand by the MB10-50 (Mevex corporation LTD., Canada) with 10 MeV Electron Beam Energy, 5,000 μ A Beam Current, 560 Pulse Repetition Frequency and 50 kw Beam Power capability at different doses of 0, 2, 3.5, and 5 kGy at room temperature. After irradiation, the irradiated samples were analyzed for quality characteristics compared to non irradiated samples as follows:

3.2.1 Physical properties: The color values L* (lightness), a* (redness) and b* (yellowness) of the products were measured using a chromameter (Minolta CR-10). Firmness was measured by the modified method of Obuz, & Dikeman (2003) using a Penetrometer by measuring the distance (in millimeters) of a plunger tip penetrating into the products.

3.2.2 Chemical properties: The water activity (a_w) of the products was measured by a water activity meter (Aqualab model Series 3TE, Decagon, USA). The nutritional compositions were analyzed by proximate analysis (AOAC, 2000). Rancidity was measured by analysis of TBA value (2-thiobarbituric acid) as the method of Wrolstad (2005). In addition, biological active compounds and activities were analyzed as follows: total phenolic content (TPC) as the method of Singleton, & Rossi (1965); ACE inhibitory activity as the method of Li et al. (2005); and GABA as the method of Kitaoka, & Nakano (1969).

3.2.3 Microbial analysis: The microbial analysis was done after irradiation. The total aerobic bacteria and yeast & mold were determined by USFDA/CFSAN/BAM methods and indicated as logCFU/g. Clostridium perfringens (pathogenic spore-forming bacteria) was determined by the AOAC method (2000) and indicated as CFU/g. Using a Stomacher laboratory blender, each sample was decimal diluted tenfold with sterile distilled peptone water (0.1%) before homogenizing. The diluent was used for subsequent ten-fold dilution. For total aerobic bacteria, pour plating of 1.0 ml of each dilution was carried out on plate count agar (PCA) and incubated for 24-48 h at 30±1°C. On dichloran rose-bengal chloramphenicol (DRBC) agar, spread plating of 0.1 ml of each dilution was done for molds and yeast. For *C. perfringens*, spread plating of 0.1 ml of each dilution was carried out on trypticase sulfite neomycin (TSN) agar pour over cover the surface with TSN agar and incubated for 24-48 h at $46\pm1^{\circ}$ C.

3.2.4 Sensory evaluation

The products after irradiation were sensory tested by 10 trained panels using 9-point hedonic scale compared to the non irradiated products.

3.3 Shelf-life study

The shelf-life of the products was determined using the method of Mizrahi (2004) by subjecting the products to accelerated aging at 40 and 50°C. The products were taken during the shelf-life study every nine days for microbiological analysis (*Clostridium perfringens*) and rancidity assessment (TBA content). The temperature-accelerating factor or temperature quotient, Q_{10} , and Q_1 were calculated as follows.

 Q_{10} = Shelf life at 40°C (days)/ Shelf life at 50°C (days), $Q_1 = Q_{10}^{0.1}$

3.4 Statistical analysis

Each analysis was run in triplicate. The information was shown as mean \pm standard deviation. Statistical analysis was conducted using SPSS 11 for Windows (SPSS Inc., USA). Analysis of Variance (ANOVA) was performed for each

Table 1 Effect of EBI dose on product quality

parameter using a completely randomized experimental design. Using Duncan's New Multiple Range Test (DMRT), a difference in results was deemed significant at p < 0.05.

4. Results and Discussion

4.1 Effect of gamma radiation dose on product qualities

EBI had no effects on physical and chemical properties of plant-based hard-boiled egg and plantbased burger patties, which differed from cobalt-60 irradiation in our previous study (Puangwerakul et al., 2023a; Puangwerakul et al., 2023b). These results were consistent with many reports showing that EBI was superior to gamma ray at the same dose, with fewer off-odors and lower peroxide values and thiobarbituric acid values (Yu et al., 2022; Zheng et al., 2022; Kong et al., 2017). The effect of different electron beam irradiation (EBI) doses on product qualities was shown in Table 1.

According to microbial destruction, all microorganisms could be destroyed by radiation at doses of 3.5 kGy and higher. However, following the 2 kGy treatment, total bacteria including yeast and mold counts, were dramatically reduced, reaching 3 log cycles and 2 log cycles, respectively. On *Cl. perfringens*, EBI needed to be more clearly effective. The outcome demonstrated that this bacterium nearly vanished following 3.5 kGy of ionized radiation treatments. This observation might be explained by the target theory of different sensitivity of the microbial populations to the EBI (Moreira, 2006).

Product		Plant-based h	ard-boiled e	gg	Plant-based burger patties				
qualities	0kGy	2kGy	3.5kGy	5kGy	0kGy	2kGy	3.5kGy	5kGy	
Physical ^{ns}									
L*	81.80±1.62	81.10±1.60	79.80±1.65	78.10±1.70	55.70±1.27	56.30±1.15	55.80±1.32	56.10±1.00	
a*	7.52±0.64	7.58±0.55	7.60±0.57	7.60±0.40	6.10±0.10	6.20±0.22	6.30±0.15	6.10±0.12	
b*	13.80±0.22	13.60±0.25	13.70±0.27	13.30±0.21	23.40±1.21	22.40±1.20	23.00±1.25	23.10±1.22	
Firmness (mm)	101.0±2.82	100.0±2.90	102.0±2.85	101.0±2.85	60.20±2.05	60.80±2.12	61.00±2.50	64.10±2.00	
Chemical ^{ns}									
Water activity	0.780 ± 0.01	0.780 ± 0.02	0.780 ± 0.00	0.780±0.00	0.972±0.00	0.975±0.00	0.971±0.00	0.970±0.00	
Moisture (%)	9.99±0.17	9.97±0.15	9.89±0.20	9.64±0.18	52.44±0.26	52.80±0.30	52.16±0.28	52.38±0.27	
TBA (mmole/kg)	28.40±1.50	28.30±1.42	29.10±1.34	28.40±1.38	31.18±1.54	30.12±0.96	30.05±1.02	30.15±0.58	
Microbial (^{1,2} logCF	U/g, ³ CFU/g)								
¹ Total pacteria	4.16±0.12a	1.02±0.03b	Ob	Ob	4.62±0.50a	1.20±0.02b	Ob	Ob	
² Yeast&Mold	3.32a±0.07a	1.28±0.03b	0b	Ob	3.14±0.10a	1.54±0.05b	0b	Ob	
³ Cl.perfringens	0.66±0.05a	0.60±0.05a	0b	0b	0.80±0.02a	0.60±0.20b	0b	Ob	

The means in each row with a different letter differ significantly (p < 0.05) Superscript ns means not significant ($p \ge 0.05$)

Attribute	Plant-based hard-boiled egg				Plant-based burger patties				
	0kGy	2kGy	3.5kGy	5kGy	0kGy	2kGy	3.5kGy	5kGy	
Appearance ^{ns}	6.14±0.18	6.10 ± 0.20	6.08 ± 0.22	6.07±0.23	6.55 ± 0.20	6.60 ± 0.18	6.61±0.22	6.58±0.23	
Texture ^{ns}	6.01±0.28	6.05±0.93	6.02 ± 0.40	6.01±0.51	7.65 ± 0.70	$7.60{\pm}0.72$	$7.50{\pm}0.60$	7.60 ± 0.55	
Color ^{ns}	6.52±0.22	6.58±0.25	6.43±0.25	6.41±0.20	7.64±0.35	7.63±0.28	7.65 ± 0.32	7.62±0.34	
Aroma ^{ns}	6.12±0.28	6.15±0.25	6.10±0.27	6.18±0.25	7.57±0.30	7.58±0.29	7.56±0.32	7.52±0.20	
Tastens	6.04±0.65	6.00 ± 0.40	6.01±0.45	5.98 ± 0.40	6.60±0.22	6.57±0.16	6.60±0.20	6.62±0.18	
Overall liking ^{ns}	6.05±0.57	6.02±0.54	6.03±0.65	6.02±0.40	6.41±0.18	6.43±0.10	6.40±0.15	6.41±0.20	

Table 2 Effect of EBI dose on sensory evaluation

Superscript ns means not significant $(p \ge 0.05)$

Table 3 Chemical composition of product treate	d with 3.5 kGy compared with untreated products.
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Idama (plant-based l	nard-boiled egg	Plant-based burger patties			
Items (unit/100g)	Untreated	Treated	Untreated	Treated		
Protein (g) ns	8.18±0.15	8.14±0.12	17.0±0.25	17.1±0.10		
Lipid (g) ^{ns}	1.51±0.10	1.50±0.15	1.00±0.15	1.00±0.05		
Fiber (g) ^{ns}	7.35±0.16	7.30±0.12	6.00±0.10	6.05±0.03		
Ash (g) ^{ns}	2.05±0.05	2.10±0.05	3.40±0.15	3.55±0.54		
Carbohydrate (g) ns	70.41±1.12	70.38±1.20	21.4±2.46	23.4±2.72		
Polyphenol (mgGAE/100g) ^{ns}	63.54±0.70	63.16±0.65	256±10.2	250±15.8		
ACE inhibitory (%) ns	44.05±1.51	44.00±0.90	84±2.50	83±2.24		
GABA (mg) ns	12.51±1.25	12.02±1.20	22±1.31	22±1.34		

Superscript ns means not significant $(p \ge 0.05)$

4.2 Sensory evaluation of the products

The sensory acceptance test was conducted to assess a potential sensory alteration in relation to the various radiation dosage responses. The results (Table 2) showed that EBI did not affect the sensory attributes of the plant-based hard-boiled egg and plant-based burger patties. This investigation showed that using EBI had a negligible impact on the sensory qualities of plant-based food. Low-dose EBI of meat products, according to Arvanitoyannis et al. (2009), low-dose EBI of meat products also had no effect on the meat's nutritional values or quality.

4.3 Nutritional compositions, TPC, GABA content, and ACE inhibitory

The results of the proximate compositions, TPC, GABA content, and ACE inhibitory of the products were compiled in Table 3. It proved that the electron beam at 3.5 kGy did not change the nutritional value, including phenolic compounds, GABA content, and ACE inhibitory peptide. A number of studies indicated that doses up to 10 kGy of EBI did not significantly affect the physicochemical properties of protein, antioxidant activity as well as phenolic compounds (Zhang et al., 2023; Han et al., 2023; Rodrigues et al., 2021).

For the effect on protein, Zhang et al. (2022) reported that high dose of EBI at 10 kGy was required to change the secondary structure of okara protein polypeptide chain from α -helix to β -sheet while dose of EBI at level at 30 KGy was required to destroy α -helix in rice protein (Zhang et al, 2020) Therefore, using dose at 3.5 kGy in this study had no effect on ACEI peptide, which is an oligopeptide consisting of only 4 amino acids, including GABA which is only an amino acid. For the effect on total phenolic compounds, Han et al. (2023) mentioned that EBI at doses of 5-20 kGy did not significantly affect the phenolic compound content in peanut shells. Rodrigues et al. (2021) mentioned that EBI at doses of 10 kGy did not significantly affect the phenolic compound content in koji-berry. Although the samples used for comparison were different, it could be taken as a guideline those changes in the number of bio-functional compounds would occur only in the case of using high dose of EBI. The results were in agreement with the results of many reports that mentioned the little effect of EBI on nutritional composition and functional properties in food (Feng et al., 2019; Bhat et al.,

2008; Carocho et al., 2013a; Carocho et al., 2013b; Carocho et al., 2013c). According to Feng et al. (2019), vacuum packing of raw ground beef with electron beam irradiation (4.5 kGy) did not affect the meat's physicochemical properties. Therefore, using dose at 3.5 kGy for plant-based products was reasonable and would not be affected by EBI.

4.4 Shelf-life study of the products

Plant-based hard-boiled egg and plant-based burger patties products had low initial lipid content and were packed under vacuum condition. Therefore, it was not conductive to the rancidity of lipid caused by the reaction between polyunsaturated of fatty acid and oxygen (Manzocco et al., 2020). This is why no TBA value change was observed throughout the accelerated storage period. EBI did not affect on the rancidity like gamma irradiation. Compared

with the effect of low dose gamma irradiation on the shelf life of ready to eat plantbased satay chicken in our previous report (Puangwerakul et al., 2023a; Puangwerakul et al., 2023b), EBI did not affect on the rancidity like gamma irradiation. Additionally, since EBs use electricity rather than radioactivity to create radiation, it is considered a safer preservation method than gamma irradiation (An et al., 2017). However, because EBs can only penetrate 8 cm into food, this treatment has only been applied to a few foods, including thin burger patties with a maximum thickness of 3 cm and half of hard-boiled egg boiled eggs. Irradiation (3.5 kGy) significantly destroyed the initial microbial level immediately irradiation and during storage at 40 and 50°C for 117 and 63 days, respectively. No viable cells of *Clostridium perfringens* were detected, as shown in Table 4. However, during storage by the method of accelerated shelf-life testing of products at 40° and 50 °C, *Cl. perfringens* was found in plant-based products at 40°C on day 117 and at 50 °C on day 63 since storage temperature after irradiation supports the recovery of *Cl. Perfringens* injured spores to normal cells along with helping to stimulate the growth of this bacteria. Clifford, & Anellis (1975) also indicated that storage temperature after irradiation (gamma ray from cobalt 60) affected the recovery of *Cl. perfringens*.

In this study, Clostridium perfringen, a pathogenic spore-forming bacteria, represents food safety. It can contaminate plant-based food products through interaction with ingredients, raw materials, and manufacturing equipment. Although the amount found is lower than the microbiological standard limit for ready-to-eat foods, which is not more than 100 CFU/g, it can be used as an indicator to determine the accelerated end of the shelf life of products. Therefore, using the calculated Q10, the predicted shelf life of plant-based hard-boiled egg and plant-based burger patties was 217 days, equivalent to 7.25 months. These results were similar to the shelf life of the whole plant-based hard-boiled egg and satay chicken products previously reported by Puangwerakul et al. (2023a; 2023b).

Days	Plant-based hard-boiled egg					Plant-based burger patties					
of	40°C		50°C				40°C	50°C			
storage	Cl.	TBAns	Cl.	TBAns		Cl.	TBA	Cl.	TBA		
D0	0±0.00b	28.41±1.59	0±0.00c	28.40±1.50		0±0.00b	30.10±0.95b	0±0.00c	30.10±0.95b		
D9	0±0.00b	28.10±1.54	0±0.00c	28.46±0.97		0±0.00b	30.12±01.02b	0±0.00c	30.97±1.25b		
D36	0±0.00b	29.12±1.50	0±0.00c	30.04±1.42		0±0.00b	32.12±1.04b	0±0.00c	32.65±1.48ab		
D54	0±0.00b	30.41±1.60	0±0.00c	29.04±1.58		0±0.00b	31.14±1.20b	0±0.00c	32.26±1.05ab		
D63	0±0.00b	30.06±1.59	0.8±0.10b	29.82±1.62		0±0.00b	35.00±0.45a	1.80±0.30a	35.06±0.52a		
D90	0±0.00b	30.10±1.61	0.6±0.15b	30.01±1.50		0±0.00b	34.90±1.02a	2.00±0.20a	35.06±0.50a		
D117	1±0.00a	30.23±1.49	1.7±0.15a	30.11±0.53		2±0.00a	35.06±1.65a	1.10±0.15b	35.05±0.94a		

Table 4 Changes in microbes (CFU/g) and TBA (mmole/kg) of product treated with 3.5 kGy on storage.

The means in each column with a different letter differ significantly (p < 0.05) Superscript ns means not significant ($p \ge 0.05$)



Figure 1 Ready-to-eat plant-based hard-boiled egg and plant-based burger patties in vacuum packaging with different EBI dose at 0, 2, 3.5 and 5 kGy.

5. Conclusion

Ready-to-eat plant-based hard-boiled egg and burger patties packed in vacuum sealed MITSUBISHI DIAMIRON M film by Thermoform Machine treated with 3.5 kGy EBI were examined for quality, safety, and stability for shelf life of 217 days or 7 months. The innovative products are distinct from existing plant-based foods in Thailand and other nations, and they have the potential to be commercialized and they are shelf-stable products. It saves cost on transportation and storage because there is no need to keep them cold.

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7. References

An, K. A., Arshad, M. S., Jo, Y., Chung, N., & Kwon, J. H. (2017). E-beam irradiation for improving the microbiological quality of smoked duck meat with minimum effects on physicochemical properties during storage. *Journal of food science*, 82(4), 865872. https://doi.org/10.1111/1750-3841.13671

- Amiri, A., Zandi, H., & Khosravi, H. M. (2019). Effect of electron beam irradiation on survival of *Escherichia coli* 0157:H7 and *Salmonella enterica* serovar thyphimurium in minced camel meat during refrigerated storage. *Journal of Food Quality and Hazards Control*, 6(4), 174-178. https://doi.org/10.18502/jfqhc.6.4.1996
- AOAC. (2000). Official Method of Analysis (17th ed.). Washington DC: Association of Analytical Chemists.
- Arvanitoyannis, I. S., Stratakos, A., & Mente, E. (2009). Impact of irradiation on fish and seafood shelf life: a comprehensive review of applications and irradiation detection. *Critical Reviews in Food Science and Nutrition*, 49(1), 68–112. https://doi.org/10.1080/10408390701764278
- Bhat, R., Sridhar, K. R., Young, C. C., Bhagwath, A. A. & Ganesh, S. (2008). Composition and functional properties of raw and electron beam irradiated *Mucuna pruriens* seed. *International Journal of Food Science* & *Technology*, 43(8), 1338-1351.

https://doi.org/10.1111/j.1365-2621.2007.01617.x

- Carocho, M., Barros, L., Antonio, A. L., Barreira, J. C. M, Bento, A., Bento, A., Kaluska, I. & Ferreira, I. C. F. R. (2013a). Analysis of organic acids in electron beam irradiated chestnuts (*Castanea sativa* Mill.): Effects of radiation dose and storage time. *Food and Chemical Toxicology*, 55, 348-352. https://doi.org/10.1016/j.fct.2013.01.031
- Carocho, M., Antonio, A. L., Barros, L., Bento, A., Botelho, M. L., Kaluska, I. & Ferreira, I. C. F. R. (2012b). Comparative effects of gamma and electron beam irradiation on the antioxidant potential of Portuguese chestnuts (*Castanea sativa* Mill.). *Food and Chemical Toxicology*, 50(10), 3452-3455. https://doi.org/10.1016/j.fct.2012.07.041
- Carocho, M., Barreira, J. C. M., Antonio, A. L., Bento, A., Kaluska, I. & Ferreira, I. C. F. R. (2012c). Effects of electron beam radiation on nutritional parameters of Portuguese chestnuts (*Castanea sativa Mill.*). *Journal* of Agricultural and Food Chemistry, 60(31), 7754-7760. https://doi.org/10.1021/jf302230t
- Chamba, M. V. M., Hua, Y. & Simwaka, J. E. (2014). Comparative sensory evaluation of soy protein isolates extracted from full fat and defatted flours using natural and conventional synthetic extraction chemicals. *International Food Research Journal*, 21(1), 209-215.
- Chen, Q., Cao, M., Chen, H., Gao, P., Fu, Y., Liu, M., ... & Huang, M. (2016). Effects of gamma irradiation on microbial safety and quality of stir fry chicken dices with hot chili during storage. *Radiation Physics Chemistry journal*, *127*(10),122-126. https://doi.org/10.1016/j.radphyschem.2016. 06.022
- Choudhury, D., Singh, S., Seah, J. S. H., Yeo, D. C. L., & Tan, L. P. (2020).
 Commercialization of plant based meat alternatives. *Trends in Plant Science*, 25(11), 1055–1058.
 https://doi.org/10.1016/j.tplants.2020.08.006
- Clifford, W. J., & Anellis, A. (1975). Radiation resistance of spores of some Clostridium perfringens strains. *Applied microbiology*, 29(6), 861-863.

https://doi.org/10.1128/am.29.6.861-863.1975

- Cutrubinis, M., Chirita, D., Savu, D., Secu, C. E., Mihai, R., Secu, M., & Ponta, C. (2007). Preliminary study on detection of irradiated foodstuffs from the Romanian market. *Radiation Physics and Chemistry*, *76*(8-9), 1450-1454. https://doi.org/10.1016/j.radphyschem.2007. 02.049
- E-Beam Services, Inc. (2014). Why do companies choose electron beam irradiation to sterilize their products?. Retrieved 18 August 2023 from https://ebeamservices. com/blog/companies-choose-electronbeam-irradiation-sterilize-products
- Feng, X., Jo, C., Nam, K. C. & Ahn, D. U. (2019). Impact of electron-beam irradiation on the quality characteristics of raw ground beef. *Innovative Food Science and Emerging Technologies*, 54, 87-92.
- https://doi.org/10.1016/j.ifset.2019.03.010 Franck, T. (2019). *Alternative meat to become*
- S140 billion industry in a decade, Barclayspredicts. Retrieved 5 April 2020, from https:www/cnbc.com/2019/05/23/alternativ e-meat-to-become-140-billion-industrybarclays-says.html
- Han, N., Lee J. Y., Kim, M., Kim, J. K., Lee, Y. Y., Kang, M. S., & Kim, H. J. (2023).
 Effect of electron-beam irradiation on functional compounds and biological activities in peanut shells. *Molecules*, 28(21), Article 7258.
 https://doi.org/10.3390/molecules28217258
- Kitaoka, S. & Nakano, Y. (1969). Colorimetric determination of ω-amino acids. *The Journal of Biochemistry*, 66(1), 87-94. https://doi.org/10.1093/oxfordjournals.jbche m.a129124
- Kong, Q, Yan, W, Yue, L, Chen, Z, Wang, H., Qi, W., (2017). Volatile compounds and odor traits of dry-cured ham (prosciutto crudo) irradiated by electron beam and gamma rays. *Radiation Physics and Chemistry*, 130, 265–272. https://doi.org/10.1016/j.radphyschem.2016.
- Li, D. (2014). Effect of the vegetarian diet on noncommunicable disease. *Journal of Science of Food and Agriculture*, *94*(2), 169-173.

09.008

https://doi.org/10.1002/jsfa.6362.Epub2013 Oct2

Li, G. H., Liu, H., Shi, Y. H., & Le, G. W. (2005). Direct spectrophotometric measurement of angiotensin I-converting enzyme inhibitory activity for screening bioactive peptides. *Journal of Pharmaceutical and Biomedical Analysis*, *37*(2), 219-224. https://doi.org/10.1016/j.jpba.2004.11.004

Li, G. L., Zheng, X. Y., Huang, D. M., Chen, X.,

- Meng, F., & Lin, M. (2019). Effect of 60Co γ-irradiation on physicochemical properties of Adlay during storage period. *International Journal of Food Engineering*, 15(8), 1-11. https://doi.org/10.1515/ijfe-2018-0329
- Lin, M. N., Chiu, T. H., & Chang, C. E. (2019). The impact of plant-based dietary pattern on dementia risk: a prospective cohort study. *Innovation in Aging*, 3(Supp 1). Article S734.
- https://doi.org/10.1093/geroni/igz038.2691 Lung, H. M., Cheng, Y. C., Chang, Y. H., Huang,
- H. W., Yang, B. B., & Wang, C. Y. (2015). Microbial decontamination of food by electron beam irradiation. *Trends in Food Science and Technology*, 44(1), 66-78. https://doi.org/10.1016/j.tifs.2015.03.005
- Manzocco, L., Romano, G., Calligaris, S., & Nicoli, M. C. (2020). Modeling the effect of the oxidation status of the ingredient oil on stability and shelf life of low-moisture bakery products: The case study of crackers. *Foods*, 9(6), Article 749. https://doi.org/10.3390/foods9060749
- Mizrahi, S. (2004). Understanding and measuring the shelf life of food. (pp. 329). Westport: F& N Press.
- Moreira, R. G. (2006). Food irradiation using electron-beam accelerators. In Hui, Y. H. (Ed.). *Handbook of Food Science, Technology and Engineering*. Boca Raton, FL: CRC Press.
- Obuz, E., & Dikeman, M. E. (2003). Effects of cooking beef muscles from frozen or thawed states on cooking traits and palatability. *Meat Science*, 65(3), 993-997. https://doi.org/10.1016/S0309-1740(02)00314-5
- Paula, C., Carole, F., Majid, J., Salmieri, S., Desjardins, N., Sahraoui, A., & Lacroix, M. (2019). Effect of cellulose nanocrystals on

thyme essential oil release from alginate beads: Study of antimicrobial activity against *Listeria innocua* and ground meat shelf life in combination with gamma irradiation. *Cellulose*, 26(9), 5247-5265. https://doi.org/10.1007/s10570-019-02481-2

- Puangwerakul, Y. (2006). *Method of producing ready-to-eat cooking Thai rice malt*. Thai Patent No. 28281. Retrieved May.9, 2006.
- Puangwerakul, Y. (2019). Production and use of sludge from symbiotic ang-kasake sato Thai rice wine. Thai Petty Patent No.18849. Sep.17, 2019.
- Puangwerakul, Y. (2018). *Manufacture of fermented beverage from Thai rice malt.* Thai Petty Patent No.20124. Mar.28, 2018.
- Puangwerakul, Y. & Soithongsuk, S. (2017). Method for making soluble rice protein from defatted rice Bran. Thai Petty Patent No.16757. May.24, 2017.
- Puangwerakul, Y., & Soithongsuk, S. (2020). Research and development of vegan fauxmeat fortified with rice protein hydrolysate and business plan. Research Report of Ministry of Higher Education, Science, Research and Innovation (MHESI): Bangkok, Thailand.
- Puangwerakul, Y., &Soithongsuk, S. (2019). Method of production red rice yeast (angkak) and red sato Thai rice wine from non sticky rice. Thai Petty Patent No.18541. Feb.28, 2019.
- Puangwerakul, Y., & Soithongsuk, S. (2022). Innovation for plant-based foods: Allergenfree vegan meat and egg products from rice processing by-products. *Journal of Current Science and Technology*, *12*(2), 360-373. https://doi.org/10.14456/jcst.2022.28
- Puangwerakul, Y., Soithongsuk, S., Sajjabut, S., & Pewlong, W. (2023a). *Innovative nonallergenic ready to eat and easy to keep plant-based meat and egg.* [Conference presentation]. Proceeding of ASAIHL Conference., Tokyo, Japan. https://asaihl2023.soka.ac.jp
- Puangwerakul, Y., Soithongsuk, S., Sajjabut, S., Pewlong, W., Dong, L. & Si, V. (2023b). Effect of low-dose gamma irradiation on shelf life of ready to eat plant-based satay chicken [Conference presentation]. Proceeding of Thailand Research symposium 2023. (In Thai)

- Rodrigues, F. T., Koike, A. C., Silva, P. G., Negrao, B. G., Alencar, S. M., Filho, J. M., & Villavicencio, A. L. C. H. (2021). Effect of electron beam irradiation on the bioactive components of goji-berry. *Radiation Physics* and Chemistry, 179, Article 109144. https://doi.org/10.1016/j.radphyschem.2020. 109144
- Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture*, *16*(3), 144-158. https://doi.org/10.5344/ajev.1965.16.3.144
- Wrolstad, R. E. (2005). *Lipid: Handbook of Food Analytical Chemistry*. (pp. 467-564). New Jersey: John Willey and Sons.
- Yu, H., Zhang, J., Li, H., Zhao, Y., Xia, S., Qiu, Y., & Zhu, J. (2022). Effects of E-beam irradiation on the physicochemical properties of Atlantic cod (*Gadus morhua*). *Food Bioscience*, 50, Article 101803. https://doi.org/10.1016/j.fbio.2022.101803

- Zhang, Y., Kong, Y., Xu, W., Yang, Z., & Bao, Y. (2023). Electron beam irradiation alters the physico chemical properties of chickpea proteins and the peptidomic profile of its digest. *Molecules*, 28(16), Article 6161. https://doi.org/10.3390/molecules28166161
- Zhang, M., Feng, X., Liang, Y., He, M., Geng, M., Huang, Y., ... & Li, Y. (2022). Effects of electron beam irradiation pretreatment on the structural and functional properties of okara protein. *Innovative Food Science and Emerging Technologies*, 79, Article 103049. https://doi.org/10.1016/j.ifset.2022.103049
- Zheng, Q., Wang, H., Yue, L., Yan, W., Guo, H., Chen, Z., ... & Kong, Q. (2022). Effect of irradiation on volatile compound profiles and lipid oxidation in chicken powder seasoning. *Radiation Physics and Chemistry*, 191, Article 109851. https://doi.org/10.1016/j.radphyschem.2021. 109851