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Comparative Effects of HPP and Irradiation on Plant-Based Whole Hard-Boiled Eggs

Yupakanit Puangwerakul[1,*], Chitsuda Chaisakdanukul[1], Suvimol Soithongsuk[2], Surasak Sajjaut[3], and Wachiraporn Pewlong[3]

[1]Faculty of Food Technology, Rangsit University, Pathum Thani 12000, Thailand
[2]Innovative Research and Incubation of Entrepreneur Center, Rangsit University, Pathum Thani 12000, Thailand
[3]Thailand Institute of Nuclear Technology, Nakornnayok 26120, Thailand

[*]Corresponding author, E-mail: yupakanit.p@rsu.ac.th

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Abstract

This study compared the effects of high-pressure processing (HPP) and irradiation on the shelf life and quality of plant-based whole hard-boiled eggs. The objective was to determine the optimal treatment doses for microbial inactivation and shelf life, and to assess their impacts on the product's physical, chemical, and sensory properties. Irradiation treatments were applied at 0, 2, 3.5, and 5 kGy, while HPP was conducted at 0, 300, 400, and 500 MPa. Post-treatment analysis included assessments of physical, chemical, and microbial quality, focusing on *Clostridium perfringens*, and sensory evaluations using a 9-point hedonic scale. Shelf life was predicted using accelerated shelf-life testing (ASLT) at 40°C and 50°C, employing the temperature acceleration factor (Q_{10}) and temperature quotient (Q_1). Results showed that gamma irradiation at 3.5 kGy and electron beam irradiation at 5 kGy both provided a predicted shelf life of 217 days, while HPP at 500 MPa resulted in a predicted shelf life of 146 days. All treatments initially eliminated microorganisms effectively, but HPP showed potential for spore germination during extended storage. Sensory evaluations indicated that electron beam irradiation and HPP better maintained product quality compared to gamma irradiation at higher doses. This study provides valuable insights for food manufacturers seeking to enhance the safety and quality of plant-based egg alternatives using non-thermal processing methods.

Keywords**:** *plant-based egg; HPP; EBI; gamma ray; non-thermal processing; shelf life extension*

1. Introduction

Non-thermal food processing techniques, such as irradiation and high-pressure processing (HPP), have gained significant traction in recent years as alternatives to traditional thermal processing and chemical treatments (Ngamlerst et al, 2024). These methods have become increasingly popular among industrial manufacturers due to their ability to effectively destroy pathogens and spoilage microorganisms while preserving the nutritional and organoleptic qualities of food products (Indiarto, & Qonit, 2020; Schottroff et al., 2018).

Irradiation utilizes high-energy photons or charged particles to inactivate microorganisms. The safety of irradiated foods has been confirmed by international health organizations, with foods irradiated below 10 kGy deemed non-toxic (Amiri et al., 2019; Wang et al., 2023). The application of irradiation technology has expanded considerably, with more than 60 foods and food products in over 50

countries (Institute of Food Science and Technology, 2020). Ionizing radiation, particularly gamma rays and electron beam irradiation (EBI), offers advantages such as high reliability, cost-effectiveness, rapid processing, and environmental friendliness (Indiarto et al, 2023).

The microbial inactivation mechanisms of irradiation involve both direct and indirect effects. Direct effects include the denaturation of enzymes, membrane proteins, and DNA structure disruption (Sehrawat et al., 2020). Indirect effects occur through the formation of free radicals from water molecule ionization, which effectively destroy microbial cells (Shafia et al., 2019). Similarly, HPP exerts its antimicrobial effects through multiple simultaneous mechanisms, including alterations to cell membrane permeability, morphology changes, and interference with biochemical reactions and genetic mechanisms (Wickramanayake et al., 2023; Sehrawat et al., 2020).

Plant-based whole hard-boiled egg analogues have recently garnered attention as functional foods due to their nutritional profile. These products typically contain 11.55% protein, 1.55% lipid, 0.65% ash, 5.62% carbohydrate, and 1.65% dietary fiber. They are also rich sources of vitamin B and vitamin A, providing 20-50% of the Thai Recommended Daily Intake for several micronutrients (Puangwerakul et al., 2024). However, like traditional egg products, these plant-based alternatives may serve as vectors for foodborne pathogens, particularly *Clostridium perfringens* and *Bacillus cereus*.

While HPP has been studied extensively for liquid egg products (Koutsoumanis et al., 2022), limited research exists on its application to plant-based whole hard-boiled egg analogues. Similarly, there is a paucity of information regarding the effects of gamma irradiation and EBI on these novel food products (Puangwerakul et al., 2024; Puangwerakul et al., 2023).

This study aims to investigate the efficacy of non-thermal processing methods, specifically irradiation and HPP, in extending the shelf life of plant-based whole hard-boiled egg analogues. The findings will provide valuable insights for food manufacturers seeking to enhance product safety and quality, potentially opening new market opportunities and informing investment decisions in this emerging sector.

2. Objectives

The objective of this study was to compare the effects of high-pressure processing (HPP) and irradiation (gamma and electron beam) on the shelf life and quality of plant-based whole hard-boiled eggs, to determine the optimal doses of these treatments for microbial inactivation, focusing on *Clostridium perfringens*, and to assess their impact on the product's physical, chemical, and sensory properties. Additionally, the study sought to predict the shelf life of the treated products under accelerated storage conditions at 40°C and 50°C using the temperature-accelerating factor (Q_{10}) and the temperature quotient (Q1).

3. Materials and methods

3.1 Materials

Rice protein hydrolysate and rice protein isolate were kindly provided by the Innovative Research and Incubation of Entrepreneur Center at Rangsit University. Rice malt powder was purchased from the community enterprise of the Nong Sarai Farmers group. Commercial vegan egg powder was obtained from Villa Market. Dried bio-cellulose powder was also supplied by the Innovative Research and Incubation of Entrepreneur Center, Rangsit University. Dried yeast was procured from Sensient Technologies (Thailand) Ltd. Angkak red rice malt powder and salt for white eggs were purchased from Nguan Soon Pepper and Spice Co., Ltd. Gellan gum was obtained from Krungthepchemi Company Limited. All reagents were used as received.

3.2 Preparation of whole plant-based hard-boiled eggs

The preparation of plant-based whole hard-boiled eggs, each weighing 80g, followed the previously published method by Puangwerakul, & Soithongsuk (2022). The process can be briefly described as follows: The base white egg powder was prepared by mixing 35% rice protein hydrolysate, 34% rice malt powder, 17% commercial vegan egg powder, 5% dried bio-cellulose powder, 4% rice protein isolate, 3% dried yeast, 1.2% angkak red rice malt powder, 0.2% salt, and 0.6% gellan gum. Water was added at a ratio of 1:3 (w/w) to create a paste before use. The egg yolk was prepared by blending the base white egg mixture with mashed pumpkin at a ratio of 1:1 (w/w), then shaping it into small spheres. To assemble the whole egg, the white egg paste was placed in a silicone mold, and the rounded egg yolk was positioned at the center. The assembled eggs were steamed at 100°C for 15 minutes. After cooking, each sample was vacuum-sealed in food-grade nylon bags to ensure consistent treatment conditions and prevent contamination. The finished products were stored in

a refrigerator at 4±2°C until subjected to further experiments.

3.3 Gamma ray irradiation

Gamma irradiation was performed at the Thailand Institute of Nuclear Technology, Thailand. The products in the sealed laminate plastic bags were irradiated by the multipurpose gamma irradiator with Co-60 (Paul Stephens Consultancy Ltd., UK) at different doses of gamma radiation of 0, 2, 3.5, and 5 kGy at ambient temperature at an average dose rate of 2.6 Gy/hr.

3.4 Electron beam irradiation

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 μA beam current, 560 pulse repetition frequency, and 50 kW beam power capability at different doses of 0, 2, 3.5, and 5 kGy at ambient temperature.

3.5 High-pressure treatment

HPP was performed at the Thailand Institute of Scientific and Technological Research (TISTR), Thailand by the K-TKFHPP HPP MACHINE Model: HPP600MPa Serial No KF190302 (Bao Tou Kefa High Pressure Technology Co., Ltd, China) at different pressures of 0, 300, 400, and 500 MPa at 30°C for 5 minutes, using water as a pressure-transmitting medium.

3.6 Physical properties

The color values of the products, including L* (lightness), a* (redness), and b* (yellowness), were measured using a chroma meter (Minolta CR-10). Firmness was assessed using a penetrometer, following a modified method by Obuz, & Dikeman (2003). The penetration depth of the plunger tip into the products was recorded in millimeters.

3.7 Chemical properties

The water activity (a_w) of the products was determined using a water activity meter (Aqualab model Series 3TE, Decagon, USA). Moisture content was analyzed through proximate analysis according to Association of Official Agricultural Chemists (AOAC) (Horwitz, 1960) standards. Rancidity was evaluated by measuring the thiobarbituric acid (TBA) value, following the method described by Wrolstad et al., (2005).

3.8 Microbial analysis

Microbial analysis was conducted post-irradiation. Total aerobic bacteria counts were determined and reported as log CFU/g, using the methods outlined in BAM Chapter 3 (Maturin, & Peeler, 2001). Total yeast and mold counts were also determined and reported as log CFU/g, following the procedures in BAM Chapter 18 (Tournas et al., 2001). Additionally, Clostridium perfringens, a pathogenic spore-forming bacterium, was quantified and reported as CFU/g, according to BAM Chapter 16 (Rhodehamel, & Harmon, 2001).

3.9 Sensory evaluation

Sensory evaluation was conducted to assess the organoleptic properties of the treated products in comparison to non-irradiated controls. A panel of 10 trained assessors evaluated the samples using a 9-point hedonic scale, where 1 represented "dislike extremely" and 9 represented "like extremely." The evaluated attributes included appearance, aroma, taste, texture, and overall acceptability. Samples were presented in a randomized order and coded with three-digit numbers. The sensory evaluation was conducted in a controlled environment with proper lighting and temperature conditions to minimize external influences on the assessment.

3.10 Shelf life study

The shelf life of the products was determined using an accelerated storage method as described by Mizrahi (2004). Samples were stored at elevated temperatures of 40°C and 50°C to simulate extended storage periods. The presence of Clostridium perfringens, a key indicator of product safety and quality, was analyzed every 9 days throughout the storage period. The shelf life at standard storage temperature was estimated using the temperature-accelerating factor (Q_{10}) and the temperature quotient (Q1), calculated as follows:

Q_{10} = shelf life at 40°C (days)/ shelf life at 50°C (days)

$Q_1 = Q_{10}^{0.1}$

where Q_{10} represents the factor by which the reaction rate increases for a 10°C rise in temperature, and Q_1 is the factor for a 1°C temperature change. Additionally, physical, chemical, and sensory evaluations were conducted at regular intervals to comprehensively assess product quality throughout the accelerated storage period.

3.11 Statistical analysis

All experiments were conducted in triplicate, with data presented as mean ± standard deviation. Statistical analysis was performed using SPSS version 11 for Windows (SPSS Inc., USA). One-way analysis of variance (ANOVA) was employed to determine significant differences among treatments for each parameter, based on a completely randomized experimental design. Differences between mean values were considered statistically significant at $p < 0.05$. When significant differences were detected, Duncan's New Multiple Range Test (DMRT) was applied for post-hoc comparison of means.

4. Results and discussion

The products shown in Figure 1 were prepared and treated using three different non-thermal processing methods at varying intensities. From left to right, the samples include: a control (untreated), gamma irradiation at doses of 2 kGy, 3.5 kGy, and 5 kGy, electron beam irradiation (EBI) at doses of 2 kGy, 3.5 kGy, and 5 kGy, and high-pressure processing (HPP) at pressures of 300 MPa, 400 MPa, and 500 MPa. These treatments were applied to evaluate their effects on the physical and chemical properties of the plant-based whole hard-boiled eggs, allowing for a comparative analysis of how different levels of treatment impact product quality.

4.1 Effect of treatment doses on product qualities

The effects of different doses of irradiation and high-pressure processing (HPP) on the qualities of plant-based whole hard-boiled eggs are summarized in Table 1.

Gamma irradiation did not significantly affect the color (L*, a*, b*), moisture content, or water activity (aw) of the products at doses up to 3.5 kGy. However, at 5 kGy, gamma irradiation resulted in a significant increase in thiobarbituric acid (TBA) content (35.2±1.10 mmole/kg) and a decrease in firmness (103±1.0 mm), indicating a reduction in texture quality. This aligns with previous findings that higher radiation doses can alter the hardness and chewiness of proteins due to the breakdown of peptide and disulfide bonds (Mehrzadeh, & Roomiani, 2021; Riebroy et al., 2007). Despite the increase in TBA value, it remained below the threshold of 60 mmole/kg, a level at which consumers typically detect rancid odors (Mexis, & Kontominas, 2009; Puangwerakul, & Soithongsuk, 2022).

In terms of microbial destruction, gamma irradiation at doses of 3.5 kGy and higher effectively eliminated all microorganisms, consistent with reports that ready-to-eat foods can tolerate low to moderate gamma radiation doses (Kanatt et al., 2006; Song et al., 2009). Specifically, total bacteria, yeast and mold, and *Clostridium perfringens* were reduced to undetectable levels at 3.5 kGy and5 kGy.

EBI at 5 kGy also achieved complete microbial destruction without significantly affecting the physical and chemical properties of the products. This observation is supported by studies indicating that EBI causes less off-odor and has lower peroxide and TBA values compared to gamma irradiation at the same dose (Yu et al., 2022; Zheng et al., 2022; Kong et al., 2017).

HPP at 500 MPa effectively destroyed all microorganisms while maintaining the texture, flavor, appearance, and nutritional qualities of the products. This is consistent with findings that HPP preserves the sensory and nutritional attributes of food products (Farkas, & Hoover, 2000).

Based on these results, gamma irradiation at 3.5 kGy, EBI at 5 kGy, and HPP at 500 MPa were selected for further shelf life studies.

Figure 1 Plant-based whole hard-boiled eggs prepared and treated using various non-thermal processing methods and intensities. From left to right: control (untreated), gamma irradiation at 2 kGy, 3.5 kGy, and 5 kGy, electron beam irradiation (EBI) at 2 kGy, 3.5 kGy, and 5 kGy, and high-pressure processing (HPP) at 300 MPa, 400 MPa, and 500 MPa. Each sample is vacuum-sealed in food-grade nylon bags.

Table 1 Effect of gamma radiation, EBI and HPP doses on product qualities

Product qualities	Control	Gamma ray (kGy)			EBI (kGy)			HPP (MPa)		
		2	3.5	5	2	3.5	5	300	400	500
Physical										
L*^{ns}	81.47±0.43	81.05±0.41	79.77±0.74	78.93±1.09	81.45±0.32	80.88±0.59	80.10±0.63	81.18±0.95	80.88±0.62	80.72±0.80
a*^{ns}	7.52±0.57	7.58 ±0.60	7.60±0.50	7.60±0.42	7.52±0.40	7.54±0.52	7.54±0.54	7.55±0.61	7.55±0.47	7.55±0.50
b*^{ns}	13.80±0.75	13.60±0.80	13.70±0.50	13.30±0.45	13.60±0.70	13.60±0.75	13.60±0.62	13.80±0.60	13.80±0.45	13.80±0.40
Firmness(mm)	97.4±1.6^{b}	98.3±1.1^{b}	98.6±1.5^{b}	103±1.0^{a}	97.4±1.5^{b}	97.9±1.7^{b}	98.5±1.2^{b}	97.4±1.5^{b}	97.4 ±1.1^{b}	97.5±1.4^{b}
Chemical										
Water activity ^{ns}	0.76± 0.02	0.76±0.01	0.75±0.01	0.73±0.02	0.76±0.01	0.75±0.02	0.75±0.01	0.76±0.02	0.76±0.02	0.76±0.01
Moisture (%)^{ns}	80.80± 2.14	80.10±2.75	80.05±2.90	80.00±2.50	80.80± 2.38	80.20±3.05	80.20± .88	80.80± 2.74	80.70± 2.30	80.80± 2.08
TBA (mmole/kg)	28.4 ± 1.74^{b}	28.3±2.10^{b}	29.1±1.95^{b}	35.2±1.10^{a}	28.4 ± .12^{b}	29.1 ± 1.80^{b}	29.1 ± .67^{b}	28.4 ± 2.21^{b}	28.4 ± 1.66^{b}	28.4 ± 1.53^{b}
Microbial (^{1,2}logCFU/g, ^{3}CFU/g)										
^{1}Total bacteria	5.15 ± 0.15^{a}	3.02 ± 0.08^{c}	0.00± 0.00^{f}	0.00± 0.00^{f}	4.04 ±0.12^{b}	0.44 ±0.03^{e}	0.00±0.00^{f}	4.15 ± 0.10^{b}	2.04 ± 0.77^{d}	0.00± 0.00^{f}
^{2}Yeast&Mould	2.18± 0.20^{a}	1.40± 0.10^{b}	0.00± 0.00^{d}	0.00± 0.00^{d}	1.47± 0.03^{b}	0.28± 0.07^{c}	0.00±0.00^{d}	0.30± 0.01^{c}	0.00± 0.00^{d}	0.00± 0.00^{d}
^{3}*Cl.perfringens*	8.00± 1.50^{a}	4.50± 0.05^{c}	0.00± 0.00^{e}	0.00± 0.00^{e}	4.65± 0.05^{c}	0.00± 0.00^{e}	0.00±0.00^{e}	5.50± 0.15^{b}	1.50± 0.50^{d}	0.00± 0.00^{e}

Means in each row with different letters are significantly different (p<0.05)

Superscript ns means not significant (p≥0.05)

Table 2 Effect of Gamma radiation, EBI and HPP doses on sensory evaluation

Attribute	Control	Gamma ray (kGy)			EBI (kGy)			HPP (MPa)		
		2	3.5	5	2	3.5	5	300	400	500
Appearance^{ns}	6.30 ±0.82	6.30 ±1.06	6.10 ±0.57	6.00 ±0.67	6.20 ±0.92	6.10 ±0.74	6.10 ±0.57	6.30 ±0.95	6.30 ±0.67	6.30 ±0.82
Texture	6.20 ±0.92^{a}	6.20 ±0.63^{a}	6.30 ±0.82^{a}	4.00 ±0.47^{b}	6.20 ±1.14^{a}	6.20 ±0.63^{a}	6.20 ±0.92^{a}	6.20 ±0.92^{a}	6.20 ±1.14^{a}	6.20 ±0.63^{a}
Color^{ns}	6.50 ±0.97	6.60 ±0.97	6.40 ±1.07	6.40 ±0.84	6.50 ±0.97	6.50 ±1.08	6.60 ±0.97	6.60 ±0.84^{a}	6.60 ±0.97^{a}	6.60 ±1.07
Aroma^{ns}	6.10 ±0.74	6.20 ±0.79	6.10 ±0.88	5.40 ±0.52	6.20 ± 0.79	6.10 ±0.88	6.10 ±0.74	6.10 ±0.57	6.10 ±0.88	6.10 ±0.74
Taste	6.20 ±0.42^{a}	6.00 ±0.00^{a}	6.00 ±0.00^{a}	5.00 ±0.00^{b}	6.30 ±0.48^{a}	6.20 ±0.42^{a}	6.10 ±0.32^{a}	6.20 ±0.63^{a}	6.20 ±0.42^{a}	6.20 ±0.63^{a}
Overall liking	6.20 ±0.63^{a}	6.10 ±0.32^{a}	6.10 ±0.74^{a}	4.90 ±0.31^{b}	6.10 ±0.57^{a}	6.10 ±0.32^{a}	6.20 ±0.63^{a}	6.00 ±0.67^{a}	6.00 ±0.00^{a}	6.00 ±0.00^{a}

Means in each row with different letters are significantly different (p<0.05)

Superscript ns means not significant (p≥0.05)

4.2 Sensory evaluation results of the products

The sensory acceptance test was conducted to evaluate potential sensory changes in response to different treatment doses. The results (Table 2) demonstrated that electron beam irradiation (EBI) and high-pressure processing (HPP) did not significantly affect the sensory attributes of the plant-based whole hard-boiled eggs. Specifically, there were no significant differences in appearance, texture, color, aroma, taste, and overall liking scores between the control and the samples treated with EBI at 2 kGy, 3.5 kGy, and 5 kGy, as well as HPP at 300 MPa, 400 MPa, and 500 MPa ($p \geq 0.05$). Gamma irradiation, however, showed some significant effects on sensory attributes at higher doses. At 5 kGy, there was a noticeable decrease in texture (5.90 ± 0.42) and overall liking (4.90 ± 0.31) compared to the control (6.20 ± 0.92 for texture and 6.20 ± 0.63 for overall liking). These findings are consistent with previous studies indicating that higher doses of gamma irradiation can negatively impact the sensory quality of food products due to the breakdown of peptide and disulfide bonds, leading to changes in texture and potential off-flavors (Mehrzadeh, & Roomiani, 2021; Riebroy et al., 2007). The minimal impact of EBI and HPP on sensory attributes aligns with literature reports that low-dose EBI does not significantly affect the quality or nutritional value of food products (Arvanitoyannis et al., 2008). Similarly, HPP is known to preserve the fresh-like character of food, including appearance, flavor, texture, and nutritional qualities (Barba et al., 2017; Santhirasegaram et al., 2015; Farkas, & Hoover, 2000). These results confirm that gamma irradiation at 3.5 kGy is suitable for extending the shelf life of the products while maintaining acceptable sensory qualities. For EBI and HPP, the best doses based on microbial results were 5 kGy and 500 MPa, respectively, as these treatments effectively eliminated microorganisms without compromising sensory attributes.

4.3 Shelf life study

Accelerated shelf life testing of plant-based whole hard-boiled eggs at 40°C and 50°C is shown in Table 3. *Clostridium perfringens*, a pathogenic spore-forming bacterium, can contaminate plant-based food products through contact with raw materials, ingredients, and manufacturing equipment. The microbiological standard limit for ready-to-eat foods is not more than 100 CFU/g (Health protection agency, 2009), which serves as an index to indicate the accelerated end of shelf life of products.

For gamma irradiation at 3.5 kGy, *Clostridium perfringens* was not detected immediately after treatment. However, during storage, *C. perfringens* was found in products stored at 50°C on day 63 (0.7±0.6 CFU/g) and at 40°C on day 117 (1.5±0.5 CFU/g). This indicates that storage temperature after irradiation supports the recovery of injured spores to normal cells, consistent with findings by Clifford, & Anellis (1975). Using the calculated Q_{10}, the predicted shelf life of the plant-based whole hard-boiled eggs treated with gamma irradiation at 3.5 kGy was 217 days (7.25 months).

Table 3 Changes in *Clostridium perfringens* (CFU/g) treated with optimum doses on ASLT storage, shelf life prediction at 30°C.

Days of storage	Gamma ray at 3.5 kGy		EBI at 5 kGy		HPP at 500 MPa	
	40°C	50°C	40°C	50°C	40°C	50°C
D0	0±0.0^{b}	0±0.0^{c}	0±0.0^{b}	0±0.0^{d}	0±0.0^{c}	0±0.0^{e}
D9	0±0.0^{b}	0±0.0^{c}	0±0.0^{b}	0±0.0^{d}	0±0.0^{c}	1.7±2.1^{dc}
D36	0±0.0^{b}	0±0.0^{c}	0±0.0^{b}	0±0.0^{d}	2.5±0.6^{b}	3.3±4.2^{c}
D54	0±0.0^{b}	0±0.0^{c}	0±0.0^{b}	0±0.0^{d}	100.3±2.5^{a}	3.7±3.7^{c}
D63	0±0.0^{b}	0.7±0.6^{b}	0±0.0^{b}	1.5±1.3^{c}	100.0±2.0^{a}	7.0±1.0^{b}
D90	0±0.0^{b}	0.7±0.6^{b}	0±0.0^{b}	3.5±0.6^{b}	100.7±1.2^{a}	7.0±1.7^{b}
D117	1.5±0.5^{a}	1.7±1.2^{a}	1.8±0.00^{a}	5.0±1.0^{a}	100.0±1.0^{a}	10.0±1.0^{a}
Shelf life Prediction						
Q_{10}	1.857		1.857		4.000	
Q_1	1.060		1.060		1.148	
Shelf -life	217 days		217 days		146 days	

Means in column with different letters are significantly different (p<0.05)

For EBI at 5 kGy, *Clostridium perfringens* was not detected immediately after treatment or during storage at 40°C and 50°C up to 90 days. However, by day 117, *C. perfringens* was found in products stored at 40°C (1.8±0.0 CFU/g) and 50°C (1.5±0.1 CFU/g). This suggests that EBI effectively destroys initial microbial levels but that storage conditions can support the recovery of injured spores over extended periods. Using the calculated Q_{10}, the predicted shelf life of the plant-based whole hard-boiled eggs treated with EBI at 5 kGy was 217 days (7.25 months). This result is similar to the shelf life of whole plant-based hard-boiled egg and satay chicken products previously reported by Puangwerakul et al., (2023).

For HPP at 500 MPa, *Clostridium perfringens* was not detected immediately after treatment. However, *C. perfringens* was detected at 40°C on day 36 and continued to increase significantly over time, reaching 100.3±2.5 CFU/g by day 54. At 50°C, *C. perfringens* was detected as early as day 9 and increased to 10.0±1.0 CFU/g by day 117. This indicates that HPP, while initially effective, may stimulate spore germination and allow for microbial growth under extended storage conditions (Carlin et al., 2000; Evelyn, & Silva, 2019). The predicted shelf life using the calculated Q_{10} was 146 days (4.87 months).

The shelf life was calculated using the temperature-accelerating factor (Q_{10}) and the temperature quotient (Q1) by the method of Mizrahi (2004). The Q_{10} value represents the factor by which the reaction rate increases for a 10°C rise in temperature, while Q1 is the factor for a 1°C temperature change. From the data, the predicted shelf life of the product treated with gamma irradiation at 3.5 kGy and electron beam irradiation (EBI) at 5.0 kGy was 217 days at 40°C, which remained consistent at 50°C, resulting in a Q_{10} value of 1.0. For high-pressure processing (HPP) at 500 MPa, the predicted shelf life was 146 days. This indicates that the shelf life of the product treated with HPP is significantly shorter compared to gamma irradiation and EBI treatments.

In summary, gamma irradiation at 3.5 kGy and EBI at 5 kGy both provided a predicted shelf life of 217 days, while HPP at 500 MPa resulted in a shorter predicted shelf life of 146 days. These findings highlight the effectiveness of gamma irradiation and EBI in extending the shelf life of plant-based whole hard-boiled eggs, with EBI being a safer alternative due to its non-radioactive nature (An et al., 2017). However, the shelf life predictions were based on the Q_{10} value, which assumes a constant rate of microbial growth or inactivation with temperature changes. This assumption may not hold true for all microorganisms or under all conditions. Finally, the study did not account for potential variations in product composition, packaging, and storage environments that could impact the shelf life and microbial safety of the plant-based whole hard-boiled eggs.

5. Conclusion

The study evaluated the effects of gamma irradiation, electron beam irradiation (EBI), and high-pressure processing (HPP) on the shelf life and quality of plant-based whole hard-boiled eggs. Gamma irradiation at 3.5 kGy and EBI at 5 kGy both effectively eliminated *C. perfringens* immediately after treatment and provided a predicted shelf life of 217 days (7.25 months) under accelerated storage conditions at 40°C and 50°C. These treatments did not significantly affect the sensory attributes of the products, making them suitable for extending shelf life while maintaining product quality.

In contrast, HPP at 500 MPa, although initially effective in microbial destruction, showed a shorter predicted shelf life of 146 days (4.87 months). This was due to the recovery and growth of *C. perfringens* during storage, particularly at higher temperatures. The study highlights the superior effectiveness of gamma irradiation and EBI in extending the shelf life of plant-based whole hard-boiled eggs compared to HPP.

However, the study has limitations, including the use of elevated temperatures for accelerated shelf life testing, which may not reflect typical storage conditions. Additionally, the focus on *C. perfringens* as the sole indicator microorganism may overlook the impact of other spoilage organisms. The shelf life predictions based on the Q_{10} value assume a constant rate of microbial growth or inactivation with temperature changes, which may not hold true for all conditions. Future studies will consider a broader range of microorganisms, varying doses of treatments, and real-world storage conditions to provide more comprehensive insights into the shelf life and safety of plant-based whole hard-boiled eggs. Additionally, the effectiveness of combining methods, such as HPP-assisted natural preservatives or pressure-assisted thermal sterilization (an FDA-approved technique), will be explored. Further sensory tests of accelerated products and analyses of vitamin changes over 217 or 146 days of storage will also be conducted to ensure product quality and nutritional value.

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