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Hurdle Approach for Control of Enzymatic Browning and Extension of Shelf Life of Fresh-Cut Leafy Vegetables Using Vacuum Precooling and Modified Atmosphere Packaging: Commercial Application

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Abstract: Fresh-cut leafy vegetable has an image as a healthy, nutritious, and delicious product. However, the product still faces the challenge of quality retention and short shelf life, especially in tropical climate regions. Enzymatic browning in fresh-cut leafy vegetables is considered one of the most important attributes limiting the shelf life of the product. The hurdle approach using commercial vacuum precooling in combination with modified atmosphere packaging (MAP) as an alternative to the use of chemical preservatives to prevent enzymatic browning, an undesirable attribute that is easily detected by consumers, was investigated. The hurdle technology exhibited synergistic effects on fresh-cut lettuce, namely frillice iceberg, romaine, and red oak, in slowing down cut-surface browning, maintaining quality, delaying microbial growth, and extending shelf life of salad products at the retail level. The findings of the study verified the potential of the hurdle approach in delaying the effect of cutting as well as extending shelf life of the product stored at 4 ± 1 °C with 85% RH from three days to nine days with an additional unit cost of 1.05%. Therefore, our hurdle approach is anticipated as the practice with non-chemical and economical approach in the supply chain of the fresh-cut, leafy vegetables industry.

Keywords: vacuum cooling; modified atmosphere packaging; hurdle technology; fresh-cut; shelf life; quality; vegetables; postharvest

1. Introduction

Minimally processed or fresh-cut leafy vegetables are prepared with methods by physical modifications such as trimming, cutting, washing, rinsing, and packaging that subsequently move along the commercial processing or are stored in cold storage conditions for a short period [1]. According to their convenience and nutritional benefits, fresh-cut salads are ready-to-eat products with a growing market share and are increasingly popular with consumers. The most popular fresh-cut product is fresh-cut salads, both single and blended. For example, approximately 19.4 million Italians consumed ready-to-eat salads in 2017, so ready-to-eat salad growth is up 4% when compared with the year 2016 [2]. Lettuce (*Lactuca sativa*), known as a salad, is consumed worldwide for the purposes of promoting well-being and preventing or reducing chronic diseases. It is also known as being a good source of vitamin A, C, E, K, folate, carotenoids, and phenolic compounds especially phenolic acids and flavonoids [3]. Fresh-cut products contribute to food loss and waste of approximately 1.3 Gigatons or 33% of global food production [4], which has become an important obstacle to be concerned in the food supply chain. During preparation, the cutting process damages the plant tissues and then induces physiological responses, such as an increase in the respiration rate and ethylene production. This hastens the metabolic rate during the storage period and finally results in the stage of senescence [5]. Breakage of internal compartments allows close proximity and reaction of enzymes and substrates, such as polyphenol oxidase (PPO) and peroxidase (POD) in enzymatic browning reactions and chlorophyllase in chlorophyll degradation [6]. The release of nutrients from damage to the outer layer promotes the growth of microbiological organisms. A high respiration rate accelerates the consumption of O_2 in the packages. Then, the change of gas composition leads to the anaerobic conditions, finally resulting in an off-flavor. During transpiration, wilting is the effect of moisture loss, while perishing is caused by the condensation of water vapor. Both physiological damage and microbiological spoilage affect quality degradation, safety aspects, visual quality, and consumer acceptance [7]. Cold storage plays an important role in the preservation of fresh-cut products by slowing down temperature-dependent reactions. The recommended storage temperature for fresh-cut vegetables ranges from 0 to 5 °C, which results in lower respiration rate, enzyme activity, and microbial growth, as well as an increase in bioactive compounds [8]. Commercial vacuum precooling technology has been deployed in the production of fresh-cut vegetables instead of room cooling or forced air cooling. Based on the association between ambient pressure and the boiling point of water, when moisture evaporates from the product, the reduction of product temperature starts. When the amount of accumulated heat is equal to the latent heat of evaporation, moisture evaporates rapidly [9]. In addition to the advantages of vacuum cooling technology, namely rapid cooling, uniform cooling, and extending shelf life, the use of the combined concept for preserving the quality of fresh-cut vegetables for designing new fresh-like fruit products is a prospect of future application for quality and safety of the products. The research illustrated that vacuum cooling had an effect on PPO activity and improves the quality of a product at a comparatively lower temperature than the other techniques [10]. Modified atmosphere packaging (MAP), known as an innovative approach to maintain or improve the food quality, safety, and the shelf life [11,12], was reported to have the ability to prolong the shelf life of Chinese kale up to 11 days. It does this by maintaining the texture, flavor, and visual quality as well as preserving the nutritional compounds such as chlorophyll and vitamin C [13]. A high concentration of CO_2 (20% maximum) could preserve the chlorophyll content and decrease the membrane oxidation of mitochondria by suppressing the reactive oxygen species (ROS) generation [14], while a low concentration of O_2 could inhibit enzyme activity as well as retard the growth of microorganism [15].

This study aimed to explore the most appropriate hurdle technologies for delaying enzymatic browning and extending the shelf life of fresh-cut vegetables, employing commercial vacuum cooling in combination with modified atmosphere packaging in the supply chain of the Royal Project Foundation in Thailand (Figure 1). The study emphasizes the potential shelf-life extension of fresh-cut vegetables as they arrived at retail stores. Research examined precooling techniques as important factors combined with using modified atmosphere packaging that could delay browning, increase the retail freshness of fresh-cut salad, and maintain the highest quality with the longest practical shelf life. Designing the appropriate hurdle technologies to extend the shelf life of fresh-cut salad at the retail level was the study goal of this research.

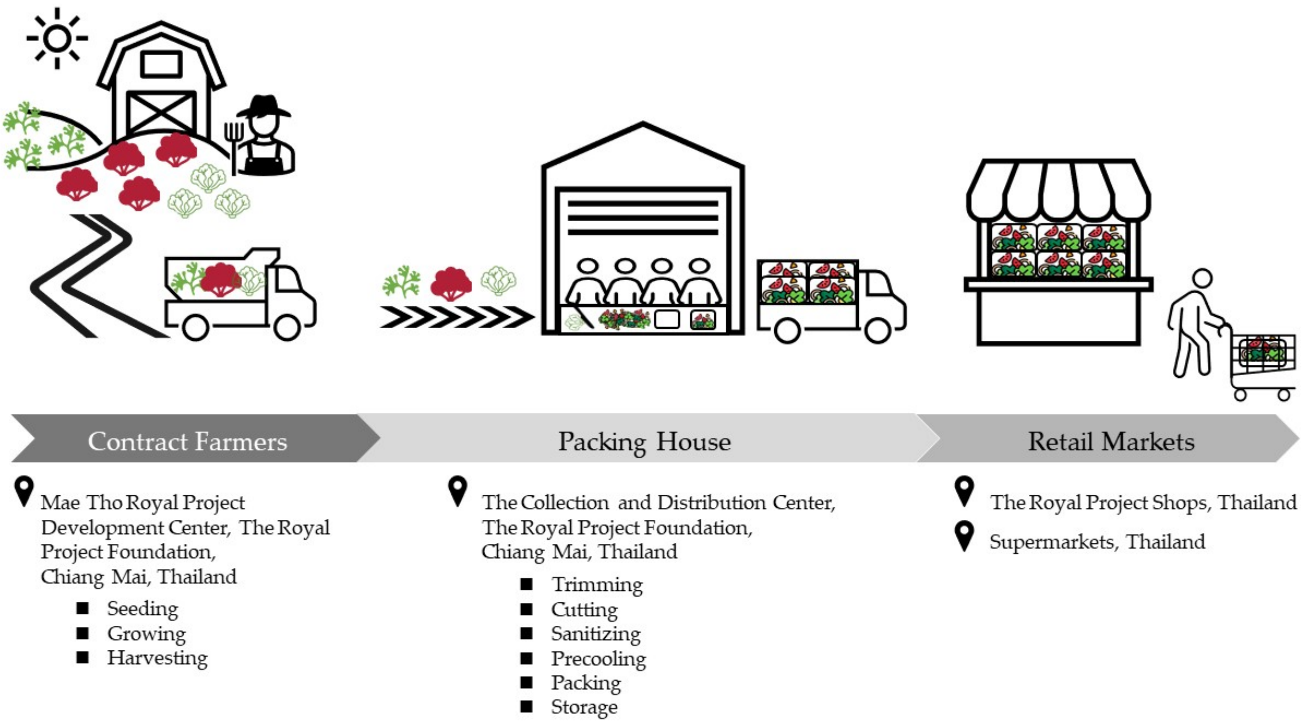

Figure 1. Three-step supply chain of fresh-cut vegetables of the Royal Project Foundation, Thailand.

2. Materials and Methods

2.1. Materials

Frillice iceberg lettuce (*Lactuca sativa* L.), romaine lettuce (*Lactuca sativa* var. *longifolia*), and red oak leaf lettuce (*Lactuca sativa* var. *crispa*) were harvested at the commercial maturity stage at the Mae Tho Royal Project Development Center, the Royal Project Foundation, Chiang Mai, Thailand. They were then transported within 3 h of harvest to the collection center at the Royal Project Foundation, Chiang Mai, Thailand.

2.2. Material Preparation, Precooling, and Packing

Three types of lettuce were prepared separately, fresh lettuces were trimmed of defects, cut into pieces 5 ± 1 cm wide, sanitized by soaking in 100 mg/mL sodium hypochlorite solution for 30 s, and spun by a salad spinner. Approximately 2.5 ± 0.1 kg of fresh-cut lettuce was packed into perforated polyethylene (PE) bags located inside polypropylene (PP) baskets.

Fresh-cut lettuces were arranged into two treatments, namely non-precooled and vacuum-precooled samples. The vacuum-precooled samples were placed in a commercial-scale vacuum precooling machine (V02 Hydro Vacuum Cooler, Hussmann, Bridgeton, MO, USA). They were subsequently precooled by decreasing the pressure within the chamber to 0.6 kPa and holding for 5, 6, and 7 min for red oak leaf, frillice iceberg, and romaine lettuce, respectively. They were controlled and determined by the timer within the machine control unit. After that, 40 g of each lettuce type within the same treatment was packed into oriented polystyrene (OPS) trays. The non-precooled samples were then covered by PP packaging (50 μm thickness) (Non/PP), while the vacuum-precooled samples were covered by PP (Vac/PP) and modified atmosphere packaging (low-density polyethylene; LDPE, 25 μm thickness, 10,000–12,000 cm^3/m^2 day oxygen transmission rate (OTR), and 5.74 cm^3/m^2 day water vapor transmission rate (WVTR)) (Vac/MAP). They were stored at 4 ± 1 °C in a simulated refrigerated retail display under lightness for 10 h and under darkness for 14 h, for further analysis. The sampling process was conducted during 7–8 a.m. each day.

2.3. Shelf Life Evaluation

Three bags of each treatment were collected randomly on the day of production and kept separately from other samples for visual evaluation daily. The visual quality of fresh-cut vegetables was evaluated by five trained panelists using a 9-point hedonic scale, where 9 = excellent, 7 = marketable, 5 = limit of marketability, 3 = limit of non-usability, and 1 = unusable [16]. The visual attributes of color, browning at the cutting edge, freshness, and overall acceptance of vegetables, were scored daily without being unpackaged. The expired date was identified when the visual evaluation score was less than 5.

The number of total aerobic bacteria, yeast, and molds contaminating fresh-cut vegetables was determined according to chapters 3 and 18 of the *Bacteriological Analytical Manual* (BAM) [17,18], respectively, while *Listeria monocytogenes* was analyzed as described in ISO 11290-1:2017 (E) [19]. The microbial loads were compared with the acceptance limit [20]. The end of shelf life of fresh-cut vegetables was determined by both visual quality and the number of microorganisms present.

2.4. Headspace Gas Analysis

The headspace gas content (O_2 and CO_2) within the packaging of fresh-cut vegetables was determined daily by injecting the headspace gas through the needle connected to the gas analyzer (F-950, Felix instruments, Camas, WA, USA) [21].

2.5. Weight Loss Analysis

Fresh-cut vegetables were weighed daily. Weight loss percentage was calculated by the difference between recent and initial sample weight [21] using an analytical balance (ELT602, Sartorius, Göttingen, Germany). Weight loss percentage was calculated as the following equation

$$\text{Weight loss (\%)} = (W_i - W_t)/W_i * 100 \quad (1)$$

where W_i is the initial sample weight (g) at day 0, and W_t is the recent sample weight (g) at day t.

2.6. Chlorophyll Analysis

The chlorophyll content of fresh-cut vegetables was estimated following the method of Witham et al. [22]. The extract of 1 g of ground frozen lettuce in 20 mL of 80% acetone (RCI Labscan, Bangkok, Thailand) was filtrated through Whatman® filter paper No.1 before volume up to 25 mL, then measured the absorbances at 645 and 663 nm by UV-Visible spectrophotometer (T60UV, PG Instruments Limited, Leicestershire, UK). The total chlorophyll content of each fresh-cut lettuce was then expressed as mg/kg fresh weight via the following equation:

$$\text{Total chlorophyll (mg/kg fw)} = [(20.2 * A_{645}) + (8.02 * A_{663})] * V/1000 * W \quad (2)$$

where V is the volume of solute (mL), and W is the weight of sample (g).

2.7. Phenolics Analysis

The method of Singleton et al. [23] was used for extracting and determining the total phenolic content of fresh-cut vegetables. Firstly, 1 mL of 10% Folin–Ciocalteu solution (Merck, Darmstadt, Germany) was reacted with 0.25 mL of methanolic lettuce extract for 3 min, and then 2 mL of 7.5% *w/v* Na_2CO_3 (Ajax Finechem Pty Ltd., New South Wales, Australia) was added. The mixture was left for 2 h before measuring the absorbance at the wavelength of 765 nm and expressed as mg gallic acid equivalents/kg fresh weight.

2.8. Enzyme Analysis

The PPO and POD activity was estimated via the method modified by Kim et al. [24]. Ten grams of the sample was homogenized with 90 mL of 0.2 mol/L sodium phosphate buffer (pH 7.0) containing 10 g of PVPP. After centrifugation (Z216MK, Hermle Labortechnik GmbH, Wehingen, Germany) at 10,000× g for 5 min, the supernatant as a crude enzyme solution was used for PPO and POD analysis. The protein concentration of each lettuce was determined via the Bradford method [25]. PPO activity was determined by spectrophotometric method. One unit of enzyme activity was determined as a 0.01/min increase in absorbance at 420 nm of the reaction between 0.8 mL of crude enzyme solution and 2.4 mL of 0.02 mol/L catechol (Merck, Darmstadt, Germany) in 0.05 mol/L sodium phosphate buffer (pH 7.0). PPO activity was expressed as enzyme units per gram of protein. One unit of POD activity was determined as a 0.001/min increase in absorbance at 470 nm of the reaction between 0.2 mL of crude enzyme solution and 2.5 mL of a mixture solution of 25 mmol/L guaiacol (Merck, Darmstadt, Germany) and 25 mmol/L hydrogen peroxide (Merck, Darmstadt, Germany) in 0.05 mol/L sodium phosphate buffer (pH 7.0). POD activity was expressed as enzyme units per gram of protein.

2.9. Statistical Analysis

Physicochemical properties, visual quality, and microbiological contamination of fresh-cut vegetables were analyzed in three replications and compared using SPSS software for Windows v.17.0 (IBM, New York, NY, USA) by one-way analysis of variance (ANOVA), followed by Duncan's multiple range test for post-hoc multiple comparisons at $p \leq 0.05$.

3. Results and Discussion

3.1. Changes in Pressure and Temperature during the Vacuum Precooling Process

The mechanism of the vacuum precooling process is described in Figure 2. In the initial step, fresh-cut lettuces were loaded into the vacuum chamber, the pressure level within was equal to the atmospheric pressure of 96 kPa. When the machine was started, the removal of air and water vapor by vacuum pump rapidly decreased the chamber pressure, while the produce temperature was slightly raised due to the accumulation of heat from the surrounding environment. On achieving the "flash point" in the seventh minute of cooling, the chamber pressure (2.48, 2.47, and 2.30 kPa, respectively) was lower than or equal to the saturated pressure (2.8–3.2 kPa) at the local temperature (23–25 °C) [26]. Water with enough accumulated heat suddenly evaporated, so the produce was automatically cooled down. The evaporation of water firstly occurs on the surface of produce, confirmed by Ozturk and Ozturk [27], who reported that the temperature at the surface of iceberg lettuce declines faster than at the center during the first 300 s of vacuum precooling. It has also been reported that temperature reduction in the leafy part of fresh-cut Brassica Chinensis is faster than in the petiole for the first 10 min of the vacuum precooling process [28]. Then, water vapor inside the produce escapes through the porous structure by pressure deficit, and the temperature distribution through the produce is finally homogenized. In the last phase, the pressure level reached the target pressure of 0.6 kPa in minutes 14, 12, and 13 of cooling. It was then maintained for 5, 6, and 7 min for red oak leaf, frillice iceberg, and romaine lettuce, respectively. A slightly declined slope of produce temperature was observed instead of the sharp one which results from a refrigeration system. The temperature of fresh-cut lettuces consisting of frillice iceberg, romaine, and red oak leaf was reduced from 21–23 °C to 4 °C within 18–20 min with an estimated energy consumption of 0.021, 0.019, and 0.021 kWh/kg, respectively. The cooling rates of fresh-cut salad consisting of frillice iceberg, romaine, and red oak leaf were 0.921, 0.955, and 0.979 °C/min, respectively. For that reason, vacuum cooling is energy efficient as well as a fast and uniform cooling method.

3.2. Shelf life Evaluation

The shelf life of fresh-cut vegetables was determined by two criteria, visual attributes and microbial load. According to the ready-to-eat salad consumer survey of Lorente-Mento, et al. [29], visual quality affected purchasing decisions at a "very much important level" were freshness, brown leaves, and exudates (70.5, 66.5, and 61.6%, respectively). The visual quality of vegetables in terms of color, browning at the cutting edge, freshness, and overall acceptance declined with storage time (Table 1). After 3 days of storage, the

Non/PP samples had significantly lower ($p \leq 0.05$) scores for browning at the cutting edge, freshness, and overall acceptance when compared with the Vac/PP and Vac/MAP samples, as the effect of the precooling method. After 7 days of storage, the combined effect of vacuum precooling and modified atmosphere packaging on the overall visual quality preserving presented a significantly higher ($p \leq 0.05$) score than the vacuum-precooled in PP packaging, similar to the study of fresh-cut rocket, lettuce, and arugula leaves packed in modified atmosphere packaging (MAP) of 5–10% O_2 [30]. Based on a 9-point hedonic scale, a cut-off score of 5 was used to identify whether the sample was accepted by the trained panelists. Browning at the cutting edge, the first attribute with a score lower than 5, was observed from 4, 8, and 10 days of storage in the Non/PP, Vac/PP, and Vac/MAP samples, respectively (Figure 3). It seems to be the most impactful problem affecting consumer acceptance and shelf life of the product [31].

Table 1. Visual evaluation of the whole packaged fresh-cut vegetables during storage at 4 °C using a 9-point hedonic scale.

Storage Time (Days)	Treatment	Visual Evaluation (Score)			
		Color	Freshness	Browning at the Cutting Edge	Overall Acceptance
0	Non/PP	9.0 ± 0.0	9.0 ± 0.0	9.0 ± 0.0	9.0 ± 0.0
	Vac/PP	9.0 ± 0.0	9.0 ± 0.0	9.0 ± 0.0	9.0 ± 0.0
	Vac/MAP	9.0 ± 0.0	9.0 ± 0.0	9.0 ± 0.0	9.0 ± 0.0
3	Non/PP	8.2 ± 0.8	7.2 ± 0.4 ^{b}	6.4 ± 0.5 ^{b}	6.2 ± 0.4 ^{b}
	Vac/PP	8.4 ± 0.5	8.4 ± 0.5 ^{a}	7.8 ± 0.8 ^{a}	7.6 ± 0.5 ^{a}
	Vac/MAP	8.2 ± 0.4	8.2 ± 0.4 ^{a}	7.8 ± 0.8 ^{a}	7.6 ± 0.5 ^{a}
7	Non/PP	Not Applicable	Not Applicable	Not Applicable	Not Applicable
	Vac/PP	6.4 ± 0.5	6.0 ± 0.7	5.2 ± 0.4	5.2 ± 0.4 ^{b}
	Vac/MAP	6.8 ± 0.8	6.6 ± 0.5	6.0 ± 0.7	6.4 ± 0.5 ^{a}
9	Non/PP	Not Applicable	Not Applicable	Not Applicable	Not Applicable
	Vac/PP	Not Applicable	Not Applicable	Not Applicable	Not Applicable
	Vac/MAP	5.6 ± 1.1	5.2 ± 1.1	5.8 ± 0.4	5.4 ± 0.9

Note: Values are the mean of three replicates ± SD. Different letters in the same column for each storage time indicate significant differences ($p \leq 0.05$).

The effect of microbial contamination on shelf life and consumer safety was also considered. Microbiological results revealed that this hurdle approach delayed microbe growth on fresh-cut salads. Table 2 shows that the numbers of total aerobic bacteria, yeast, and molds increased with storage time due to the precooling methods and storage conditions [32]. On the production date, the Non/PP samples had a significantly higher ($p \leq 0.05$) amount of total aerobic bacteria as well as yeast and molds than the others. The study of He et al. revealed that the vacuum precooling process can damage and deform *E. coli* cells [33]. However, Kongwong et al. [34], who studied the ultrastructure of baby cos, reported that no damage to the product's cells was observed after the vacuum precooling process. The lower microorganism load found in this study might be the result of good practice during the operation (to limit the initial load) and the rapid cooling characteristic of vacuum precooling (to retard microbial growth) rather than microbial damage. After 4 days of storage, there was a significantly lower ($p \leq 0.05$) number of microbial loads observed in the Vac/PP and Vac/MAP samples than the Non/PP samples, which also supported the vacuum precooling ability on controlling the growth of microorganisms. The hurdle effect of vacuum precooling and modified atmosphere packaging was then observed in Vac/MAP samples after 8 days of storage, as a significantly lower ($p \leq 0.05$) number of total aerobic bacteria than those contaminated in the Vac/PP samples. It agrees with Oliveira et al. [8], who noted that the combination of the modified atmosphere packaging (MAP) with other treatments presented better efficiency in controlling the food-borne pathogens than using it as a single treatment.

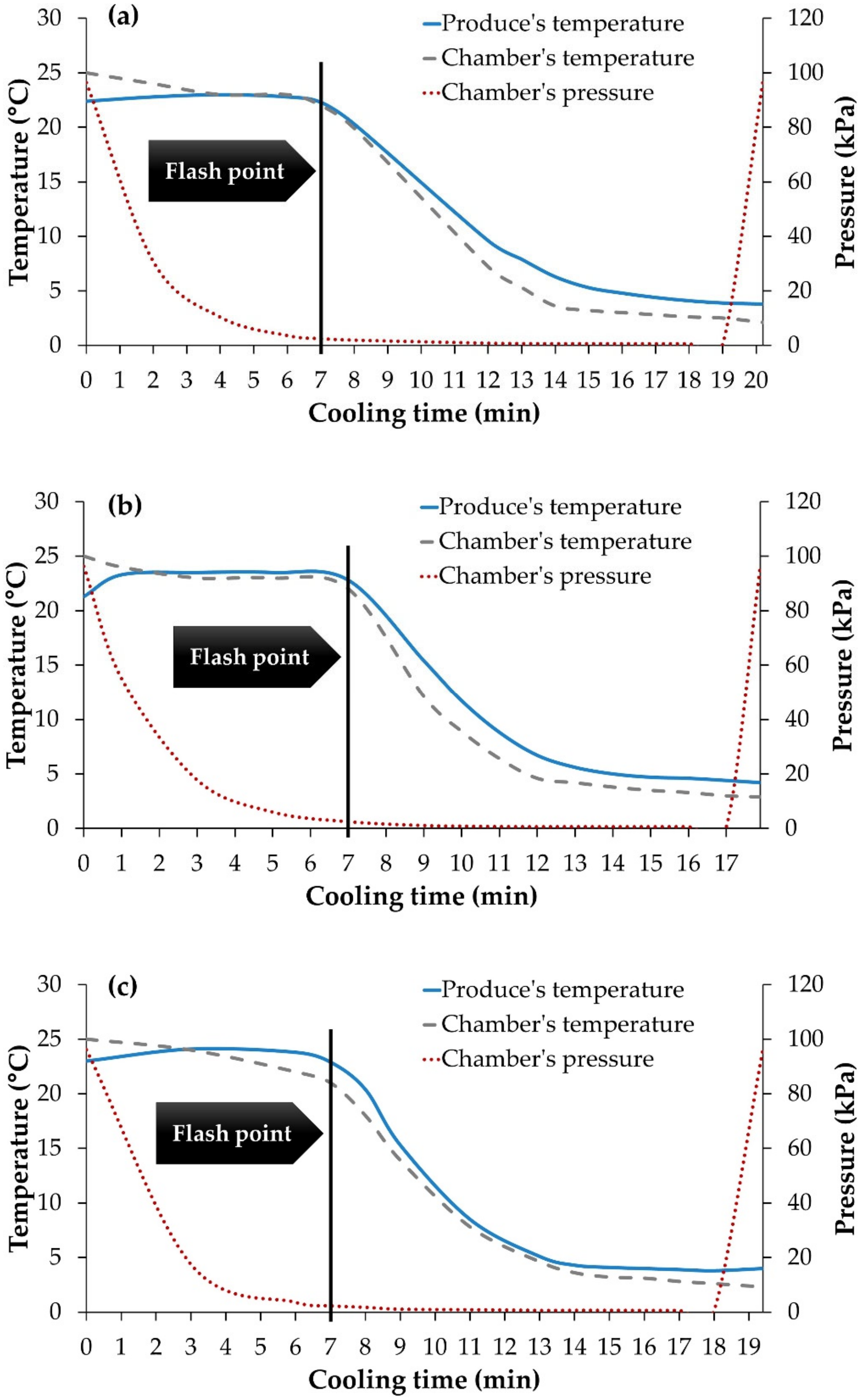

Figure 2. Cooling curve of fresh-cut frillice iceberg (**a**), romaine (**b**), and red oak leaf (**c**) lettuce, and chamber pressure and temperature changes during the vacuum precooling process.

Visual evaluation score and microbial load were displayed in Table 3. The minimum storage times which conformed to the visual and microbiological specifications were defined as the shelf life of Non/PP, Vac/PP, and Vac/MAP were 3.8 ± 0.9, 7.2 ± 0.4, and 9.0 ± 1.0 days, respectively. The longer shelf life confirmed the potential of vacuum precooling in combination with modified atmosphere packaging for prolonging the shelf life of fresh-cut vegetables, similar to the studies of baby cos [34], red romaine [35], and Chinese kale [13].

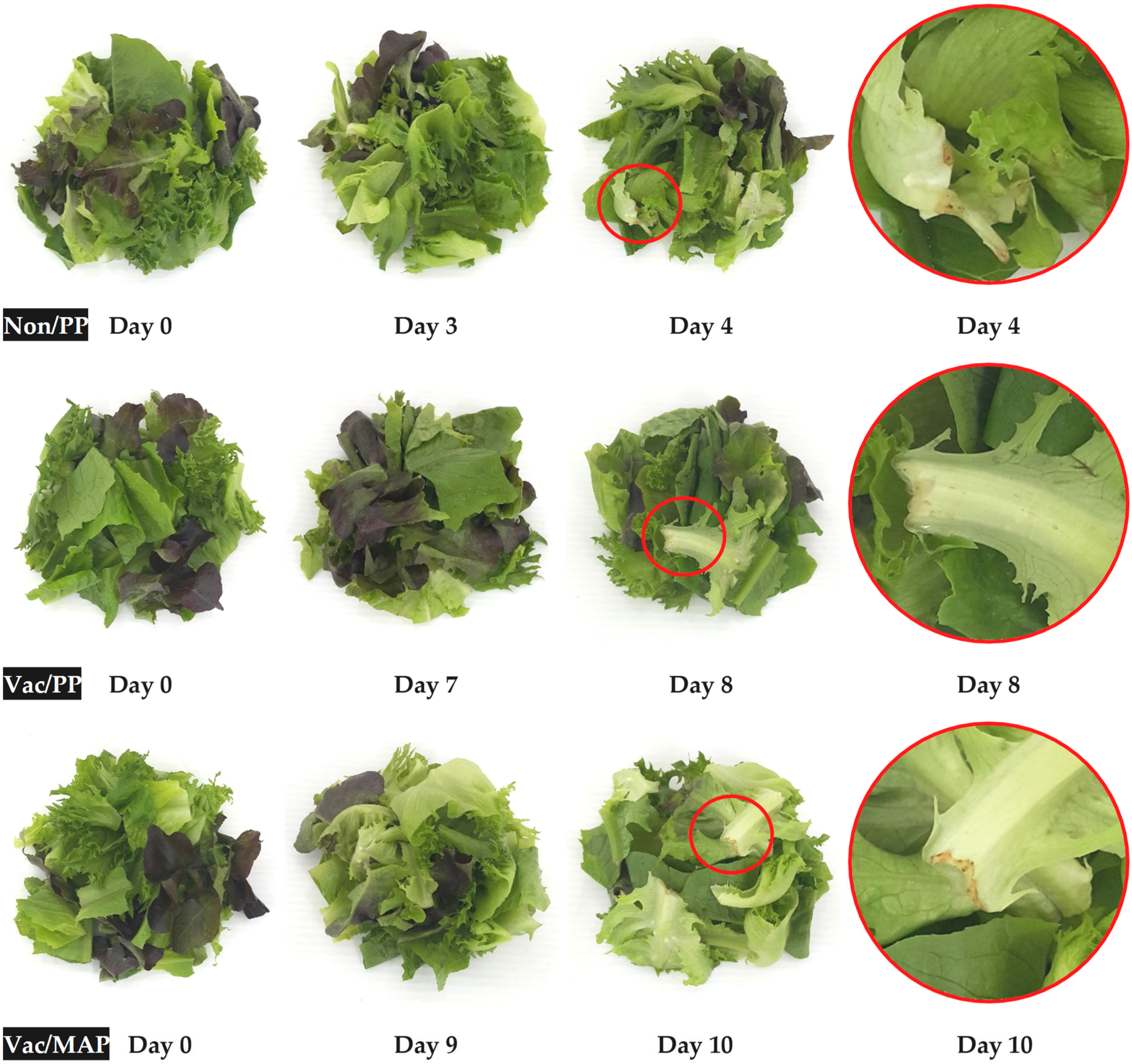

Figure 3. Images of fresh-cut vegetables during storage at 4 °C at various storage time; Non/PP at day 0, 3, 4 (**first row**), Vac/PP at day 0, 7, 8 (**second row**), and Vac/MAP at day 0, 9, and 10 (**third row**).

3.3. Headspace Gas Content

According to the respiration process, O_2 content tended to decrease as a result of O_2 usage. CO_2 production caused the increasing trend of CO_2 content during the storage time (Figure 4). Modified atmosphere packaging with higher gas permeability than PP packaging could maintain the headspace gas content as stable since the first day of storage (approximately 17% of O_2 and 1% of CO_2), while the gas content within PP packaging continues to change. After 3 days of storage, the Vac/MAP samples revealed significantly higher ($p \leq 0.05$) O_2 content (17.13 ± 0.12%) but lower ($p \leq 0.05$) CO_2 content (1.24 ± 0.02%) than the Non/PP and Vac/PP samples (15.23 ± 0.68% O_2; 3.92 ± 0.30% CO_2, and 15.00 ± 0.44% O_2; 4.07 ± 0.21% CO_2, respectively), as the result of the packaging type rather than the precooling method. The results showed that the oxygen content of approximately 17% in the modified atmosphere packaging could reduce the intensity of respiration. In addition, the condensation on the inner PP film surface (low water vapor transmission rate) when stored at 4 °C caused defects of the external appearance, promoted the growth of microorganisms, and thus accelerated deterioration.

Table 2. Microbiological analysis of the whole packaged fresh-cut vegetables during storage at 4 °C.

Storage Time (Days)	Treatment	Total Aerobic Bacteria (log CFU/g)	Yeast and Molds (log CFU/g)	*Listeria monocytogenes* (/25 g)
0	Non/PP	4.02 ± 0.57^{a}	1.00 ± 0.00	Not detected
	Vac/PP	2.56 ± 0.23^{b}	Not detected	Not detected
	Vac/MAP	2.53 ± 0.20^{b}	Not detected	Not detected
4	Non/PP	4.99 ± 0.06^{a}	1.79 ± 0.10^{a}	Not detected
	Vac/PP	4.22 ± 0.07^{b}	1.30 ± 0.30^{b}	Not detected
	Vac/MAP	4.30 ± 0.10^{b}	1.10 ± 0.17^{b}	Not detected
8	Non/PP	Not Applicable	Not Applicable	Not Applicable
	Vac/PP	6.19 ± 0.43^{a}	2.86 ± 0.62	Not detected
	Vac/MAP	5.46 ± 0.43^{b}	2.34 ± 0.59	Not detected
10	Non/PP	Not Applicable	Not Applicable	Not Applicable
	Vac/PP	Not Applicable	Not Applicable	Not Applicable
	Vac/MAP	6.26 ± 0.57	2.58 ± 0.13	Not detected

Note: Values are the mean of three replicates ± SD. Different letters in the same column for each storage time indicate significant differences ($p \leq 0.05$).

Table 3. Estimated shelf life of the whole packaged fresh-cut vegetables during storage at 4 °C.

Criteria	Specification			Shelf Life (Days)		
				Non/PP	Vac/PP	Vac/MAP
Visual quality						
Color (score)		≥5.00		5.8 ± 0.4^{c}	7.8 ± 0.4^{b}	9.2 ± 0.8^{a}
Freshness (score)		≥5.00		5.6 ± 0.5^{c}	7.8 ± 0.4^{b}	9.0 ± 1.0^{a}
Browning at the cutting edge (score)		≥5.00		3.8 ± 0.9^{c}	7.2 ± 0.4^{b}	9.4 ± 0.5^{a}
Overall acceptance (score)		≥5.00		4.4 ± 0.9^{c}	7.4 ± 0.5^{b}	9.2 ± 0.8^{a}
Microorganisms	**Target**	**Tolerance**	**Unaccepted**			
Total aerobic bacteria (CFU/g)	10^5	10^6	10^8	4.3 ± 0.6^{c}	8.3 ± 0.6^{b}	10.0 ± 0.0^{a}
Yeast and molds (CFU/g)	10^3	10^4	10^5	4.0 ± 0.0^{c}	7.3 ± 0.6^{b}	10.0 ± 0.0^{a}
Listeria monocytogenes (/25 g)	Absent	Absent	10^2	4.0 ± 0.0	8.0 ± 0.0	10.0 ± 0.0

Note: Values are the mean of three replicates ± SD. Different letters in the same row for each criteria indicate significant differences ($p \leq 0.05$). Target is the best condition guideline for the manufacturing date. Tolerance is the maximum guideline for the manufacturing date. Unaccepted is the maximum guideline for the expired date. Specifications of microorganisms refer to Ragaert et al. (2010). Specification of visual quality refers to a 9-point hedonic scale, where a 5 score = limit of marketability. The expired date was identified when the visual evaluation score was less than 5.

3.4. Weight Loss Percentage

Weight loss in fresh produce is mainly caused by transpiration through apertures such as stomata, wounds, and cut edges, which are induced by structural and other external factors such as temperature, pressure, relative humidity, and air velocity [14]. Weight loss of fresh-cut vegetables tended to increase with storage time (Figure 5). After 3 days of storage, weight loss of the Vac/MAP samples (0.18%) was significantly higher ($p \leq 0.05$) than that of Non/PP and Vac/PP samples (0.04 and 0.05%, respectively). The significantly higher ($p \leq 0.05$) weight loss was also observed in the Vac/MAP samples (0.39%) when compared to the Vac/PP samples (0.13%) after 7 days of storage. These results confirmed that the weight loss percentage is related to the packaging properties [13], especially the water vapor transmission rate (WVTR). The enlargement of stomatal guard cells is affected by water potential [36]. Lower water potential than nearby cells allows water to move into a stomatal guard cell, resulting in swollen guard cells, opened stomata, and finally the release of more water. Unperforated PP packaging with a low WVTR can protect the produce inside from external factors, while vapor accumulation in the headspace lessens the pressure deficit and limits the release of vapor from the inside out. However, the maximum weight loss of the Vac/MAP samples was 0.54 ± 0.06%, lower than the 3–5% declared as the critical limit to start wilting and decaying of fresh produce [37]. Thus, the freshness of fresh-cut vegetables was shown by their high visual evaluation score.

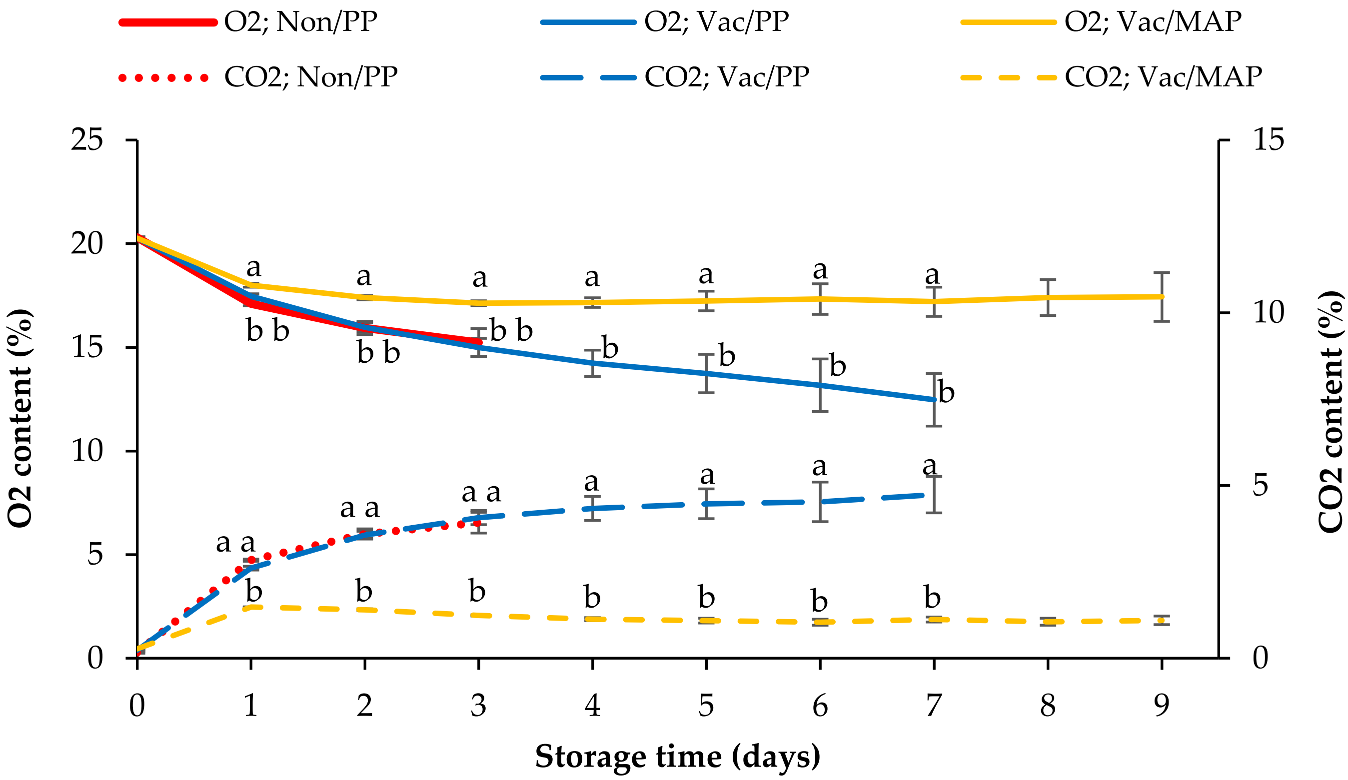

Figure 4. Changes in head space gas content of the whole packaged fresh-cut vegetables during storage at 4 °C. Error bars represent the mean of three replicates ± SD.

3.5. Chlorophyll Content

During storage, the total chlorophyll content of fresh-cut vegetables declined slightly (Figure 6a,c,e). Although decreasing chlorophyll is the effect of chlorophyll degradation, it can be retarded by chilled storage [38]. After 3 days of storage, residual chlorophyll content in the Vac/MAP samples was significantly higher ($p \leq 0.05$) than in the others. The study on ultrastructure by Kongwong et al. [34] revealed the degradation of non-precooled baby cos chloroplasts after 9 days of storage while no degradation was found in vacuum-precooled samples during the experimental period. Furthermore, Luo et al. [39] described that decreasing chlorophyll is the effect of membrane damage (vacuolar membrane, thylakoid membrane, and chloroplast envelope), which allows the chlorophyll (generally located in the closed membrane of chloroplasts) to react with chlorophyllase, resulting in chlorophyll degradation. Headspace gas content also played an important role in chlorophyll degradation. High CO_2 conditions could also damage the membrane by stimulating lipid oxidation [40], which agrees with Guo et al. [14], who reported that the modified atmosphere packaging (MAP) with lower CO_2 content than 20% helped preserve the chlorophyll content.

3.6. Total Phenolic Content

Figure 6b,d, and f reveal the increasing trend of total phenolic content found in fresh-cut vegetables. A significantly higher ($p \leq 0.05$) total phenolic content was found in romaine and red oak leaf lettuce of the vacuum-precooled samples (Vac/PP and Vac/MAP) after 3 days of storage. Phenolic compounds are a group of antioxidants that a plant synthesizes for defending itself from stress and pathogens [41]. Not only postharvest handling and fresh-cut processes, such as harvesting, cutting, packaging, transportation, and storage but vacuum-precooling processes also cause stress. To defend against stress, an automatic signal stimulates the protein synthesis of phenylalanine ammonia lyase (PAL). Subsequently, PAL activates the production of phenolic compounds from phenylalanine [7]. Conversely, phenolic compounds were diminished due to the participants as the substrate of the enzymatic browning reaction [6,35,42]. After 7 days of storage, total phenolic content in frillice iceberg and romaine lettuce of the Vac/PP samples was significantly lower ($p \leq 0.05$) than those in the Vac/MAP samples, conforming to lower enzymatic browning reaction observed in the Vac/MAP samples.

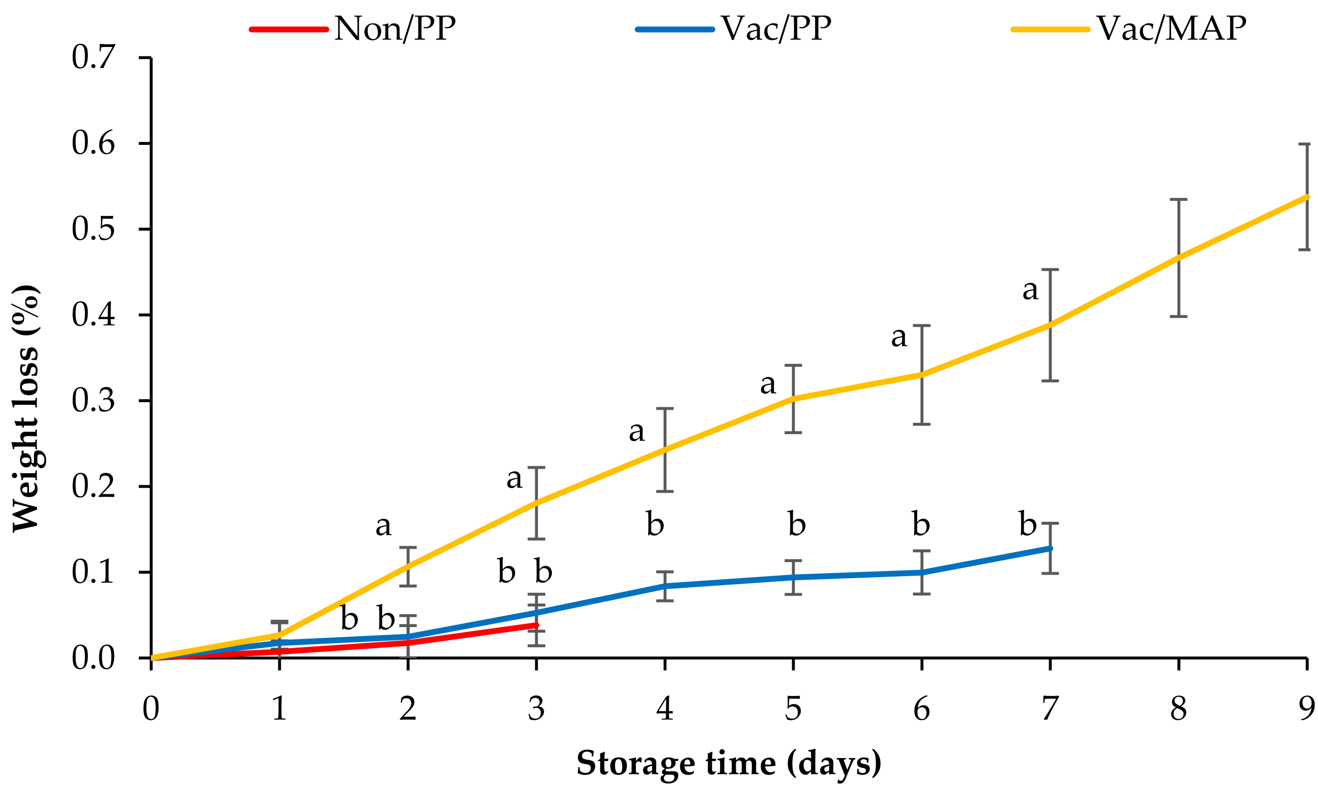

Figure 5. Changes in weight loss percentage of the whole packaged fresh-cut vegetables during storage at 4 °C. Error bars represent the mean of three replicates ± SD.

3.7. Enzyme Activity

Enzymatic browning is a major factor that contributes to the loss of quality of fresh-cut vegetables with associated changes in visual properties. According to visual quality evaluation, the appearance of a brown color at the cutting edge was easily observed and influenced consumer acceptance. The activity of PPO is the key enzyme responsible for the oxidation of phenols to quinones. Delaying enzymatic browning and color changes during the processing of fresh-cut leafy vegetables were explored using a combination of vacuum cooling and modified atmosphere packaging. The increasing trend of PPO and POD activity (Figure 7) was the result of enzymatic browning reactions. The fresh-cut process damages the natural compartments and releases phenolic compounds from the vacuole to react with enzymes in the cytoplasm; finally, a brown pigment is produced [43]. The cutting process not only plays an important role in enzymatic reactions, but it also impacts the reaction substrate by inducing the production of phenolic compounds. Figure 7a,c,e showed the increasing trend of PPO activity which was also observed in the studies of Zhan et al. and Luna et al. [43,44], related to the phenolic content as the catalyst of phenol oxidation [33]. POD, known as a wound-repair and disease-resistant enzyme, had the same increasing trend (Figure 7b,d,f) as that observed by Zhan et al. [44]. After 3 days of storage, significantly higher ($p \leq 0.05$) PPO activity of the Non/PP samples than the others were observed in frillice iceberg and red oak leaf lettuce, while a non-significantly difference ($p > 0.05$) was found after 7 days of storage. Although a greater amount of total phenolic compounds was found in the vacuum-precooled samples, the enzyme activity related to enzymatic browning reactions was lower. Application of vacuum cooling significantly ($p \leq 0.05$) affected the activity of PPO. The effect of the vacuum precooling process to slow down the reaction, as well as to preserve cell integrity were supported by the study of Kongwong et al. [34], who found that degradation of the cell wall and vacuole membrane in non-precooled baby cos promotes enzymatic browning reactions. However, maximum inhibition was observed in sample treated with both vacuum cooling process and using modified atmosphere packaging. The low oxygen and high carbon dioxide concentration with low temperature were favorable stress of lettuce, which had remarkable effect on delaying browning of fresh-cut lettuce. The findings revealed that activity of PPO was significantly reduced due to the combined effect of vacuum precooling that also resulted in improved sensory characteristics and increased total phenolic compounds as well as product shelf life.

3.8. The Possibility of Applying Vacuum Precooling and Modified Atmosphere Packaging in the Commercial Fresh-Cut Leafy Vegetables Production

The hurdle practice was more efficient than either treatment alone for maintaining physiological quality and reducing microbial growth in the supply chain process of fresh-cut leafy salad with effectiveness and energy cost efficiency. The potential of employing vacuum precooling and modified atmosphere packaging in fresh-cut production for delaying browning and prolonging the shelf life was illustrated in Sections 3.2–3.7. The additional unit cost of 0.06% and 1.05% is required for the Vac/PP and Vac/MAP treatments. Although there was a small increase in operation cost, the hurdle approach could extend the expiration date up to 133% and 200%, respectively, when compared with the Non/PP. Longer shelf life increased the opportunity for sale by extending the distance of logistics and prolonging the periods on the retail shelf life and in the consumer's refrigerator as well. In addition, products sold, food losses, and waste are reduced. Therefore, the application of this hurdle technology was appropriate and could be applied to sustainable commercial production of fresh-cut vegetables.

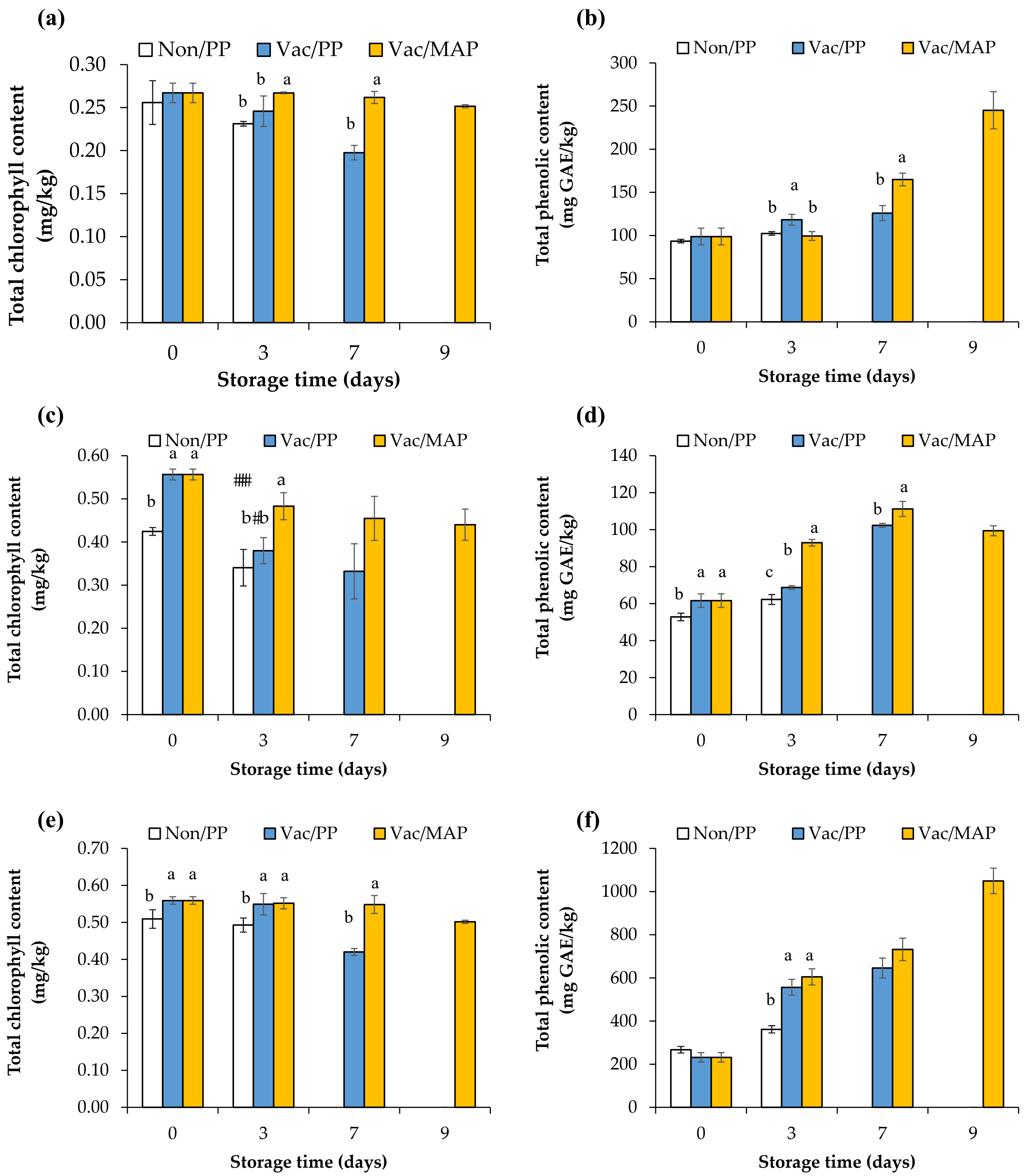

Figure 6. Changes in total chlorophyll content (**a**,**c**,**e**) and total phenolic content (**b**,**d**,**f**) of fresh-cut vegetables consisting of frillice iceberg (**a**,**b**), romaine (**c**,**d**), and red oak leaf (**e**,**f**) lettuce, during storage at 4 °C. Error bars represent the mean of three replicates ± SD.

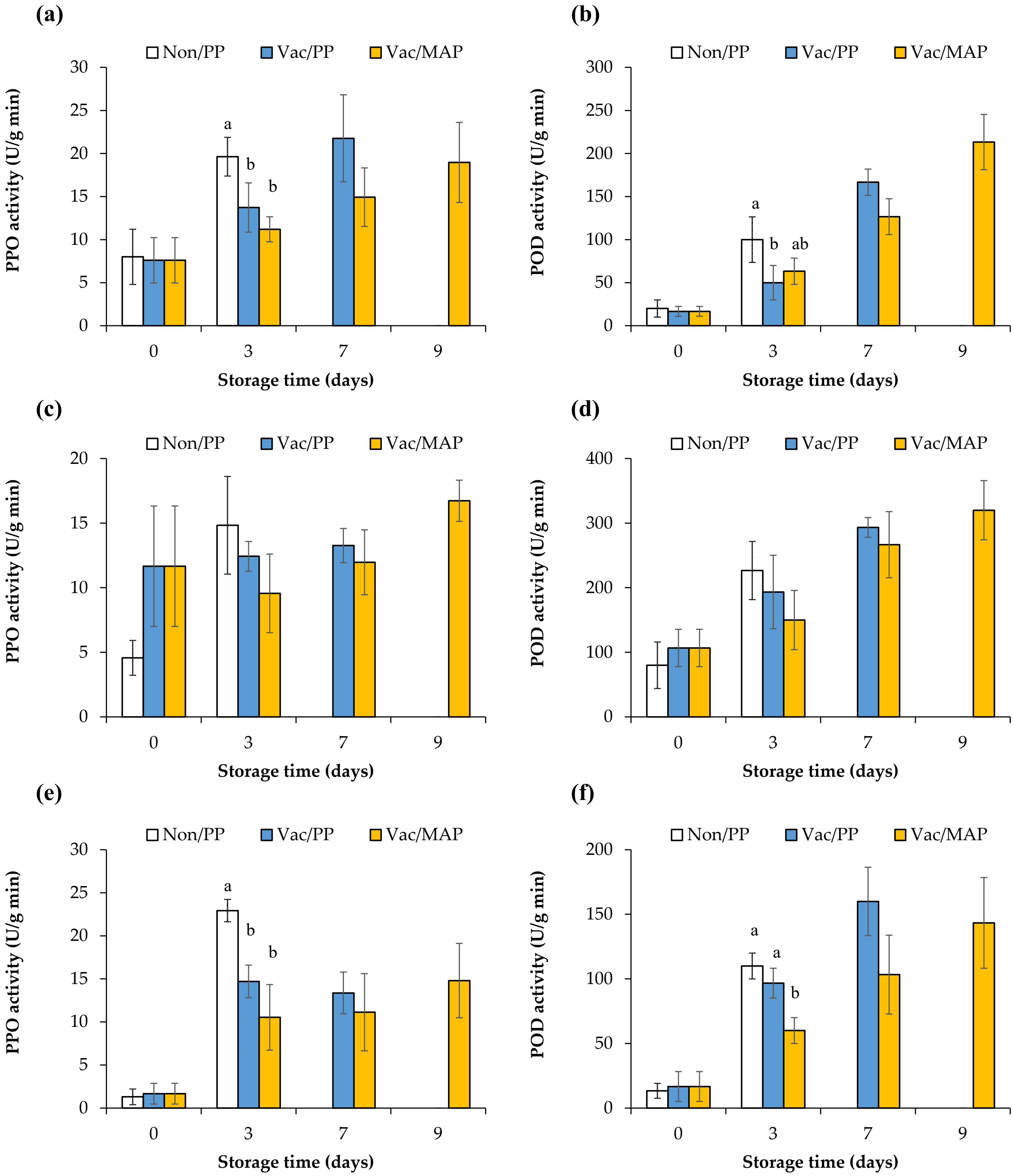

Figure 7. Changes in the activity of PPO (**a**,**c**,**e**) and POD (**b**,**d**,**f**) in fresh-cut vegetables consisting of frillice iceberg (**a**,**b**), romaine (**c**,**d**), and red oak leaf (**e**,**f**) lettuce, during storage at 4 °C. Error bars represent the mean of three replicates ± SD.

4. Conclusions

Since the browning degree was the main shelf-life indicator of minimally processed vegetables, the hurdle approach for enzymatic browning controlling and shelf life extending using vacuum precooling and modified atmosphere packaging was explored as an alternative to the use of chemical preservatives in the packing house. Synergistic effects of vacuum precooling and modified atmosphere packaging delayed enzymatic browning and prolonged shelf life of the product. Effectiveness and cost efficiency are important factors to be considered for development of an anti-browning approach. The additional unit cost of 1.05% could extend the expiration date up to 200% (from 3 to 9 days), increase the opportunity for sale by extending the distance of logistics, prolong the periods both on the retail shelf life and in the consumer's refrigerator, and reduce the occurrence of food waste as well. After taking into consideration all the potential results, the hurdle technology effects of commercial vacuum precooling combined with modified atmosphere packaging was successfully delaying enzymatic browning and prolonging shelf life of minimally processed, fresh-cut, leafy vegetables in the retail market.

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