Fresh insight: Enhancing Verdant leafy vegetable's shelf life via Modified Atmosphere Packing and storage.

ABSTRACT

This study aimed to enhance the durability of selected green leafy vegetables, including Lettuce and Palak, by manipulating influential factors such as storage conditions (Ambient (35±5°C) and Refrigerated (5±5°C)), packing substrate (low-density polyethylene and high-density polyethylene covers), and gas composition using modified atmosphere packaging. Factorial completely randomized design (FCRD) was employed, incorporating a total of 12 treatments for each crop. The evaluation primarily relied on calculating physiological weight loss. Lettuce showed optimal results with treatment T5 (0.38%) exhibiting superior performance, followed by T6 (0.45%) and T3 (0.58%) under ambient conditions. Similarly, for palak, T5 (0.18%) outperformed T3 (0.19%) and T2 (0.37%). Thus, it is advisable to adopt T5 ($R_1G_3M_1$) as the preferred treatment with T3 ($R_1G_2M_1$) as a viable alternative for ambient storage conditions. Refrigerated lettuce performed optimally with T11 (0.54%) leading the way, followed by T7 (0.56%) and T9 (0.69%). Similarly, palak exhibited superior results with T9 (0.61%) in the lead, followed by T11 (0.77%) and T7 (0.97%). Notably, produce stored under ambient conditions had a relatively short shelf life of approximately 5 days, while refrigeration extended it to 15 days, after which a decline in quality was observed across all treatments. Therefore, refrigerated produce has an extended shelf life, with T9 ($R_2G_2M_1$) followed by T7 ($R_2G_1M_1$) yielding highly favorable outcomes.

Key words: Green leafy vegetables, packing substrate, modified atmospheric packing, gas composition and longevity.

1. INTRODUCTION

The green leafy vegetables (GLV) employed in this study encompassed Lettuce and Palak carefully chosen for their discernible attributes, spanning nutritional value, export potential, and herbal properties. It is widely acknowledged that green leafy vegetables serve as substantial contributors to dietary fiber and a plethora of essential nutrients, including vitamins and minerals. They are hailed as a beacon of hope in addressing the issue of malnutrition in developing nations such as India, owing to their remarkable abundance of vital vitamins such as ascorbic acids, riboflavin, folic acid, and beta-carotene.

Lettuce, scientifically referred to as *Lactuca sativa*, belongs to the Asteraceae family. This temperate salad crop holds significant market value and is characterized by its shallow root system, rendering it highly susceptible to acidic soil conditions. Lettuce is renowned for its abundant reserves of lutein, dietary fiber, Vitamin A, Vitamin C, Vitamin Fe, and folate. The present study focuses specifically on the green leaf lettuce, known for its loose-leaf structure. This variety of lettuce has been reported to contain 0.4 $\mu g\ g^{-1}$ of folate, 92 $\mu g\ g^{-1}$ of Vitamin C, and 2.2 $\mu g\ g^{-1}$ of Vitamin E per unit of fresh weight [1,14].

Palak, scientifically known as *Beta vulgaris var. bengalensis* belonging to the Chenopodiaceae family, is a versatile pot herb that thrives in both tropical and sub-tropical climatic conditions. It stands as an excellent choice for a green leafy vegetable in kitchen gardens, as it fulfils the requirements of nutritional security while demanding minimal maintenance. In terms of nutritional composition, palak boasts noteworthy quantities of essential components, including 469 µg of Vitamin A, 28 µg of Vitamin C, 2 g of Protein, 73 mg of Calcium, and 1.14 mg of Iron per 100 g of fresh weight. The study incorporates all green variety of palak, which have been reported to contain 26 mg of ascorbic acid, 22.6 mg of carotenoids, and 2.7 mg of β-Carotene in 100 g of palak [2].

Typically, green leafy vegetables exhibit a highly perishable nature, with a relatively short shelf life of merely one to two weeks. Consequently, safeguarding the longevity of these produce items becomes a critical endeavour to mitigate nutritional loss. Notably, Lettuce, being crops oriented towards export, necessitate meticulous packaging measures to effectively extend their shelf life. Therefore, the judicious selection of appropriate packing materials, optimal storage conditions, and meticulous management of gas composition assume paramount importance in protracting the shelf life of these valuable commodities.

Modified Atmosphere Packaging (MAP) encompasses the deliberate control or alteration of the atmosphere enveloping a product within packaging composed of diverse combinations and types of films [16]. In the realm of active MAP implementation, it was noted that application of a gas mixture comprised of approximately 88% N_2, 2% CO_2, and 15% O_2 to finely chopped parsley, maintaining a temperature of 5°C. This proactive MAP strategy effectively extended the parsley's storage period by a noteworthy 6 days. These findings underscore the potential of MAP techniques to positively influence the quality and longevity of parsley, thus presenting valuable insights for enhancing post-harvest preservation practices [18]. Pertinently, studies conducted on parsley have exhibited intriguing outcomes: instances where pigmentation reduction in leaves was notably mitigated in a Controlled Atmosphere containing 10% O_2 and 10% CO_2, as opposed to when exposed to ambient air [17].

2. MATERIALS AND METHOD

2.1 Plant material and treatment

The conducted experimental endeavor took place within the esteemed premises of the Department of Food Processing and Engineering, situated within the distinguished Tamil Nadu Agricultural University. The principal objective of this undertaking was to meticulously assess the temporal longevity and preservation efficacy of GLV cultivated within an open field environment. Upon harvest, the botanical specimens underwent a meticulous aqueous washing, meticulously purging any extraneous particulate matter entangled within the intricate folds of their vibrant foliage. This hygienic purification process was further complemented by a thorough ablution, carefully executed with a gentle cloth, ensuring the complete eradication of residual impurities. The conventional washing procedure, utilizing tap water, showcased remarkable efficacy by successfully eliminating approximately 92.4% of the microbial population residing on lettuce leaves, commonly referred to as lettuce leaf microflora [3]. Subsequently, precise quantities of 250 grams of GLV were meticulously apportioned for packaging within each variant of the designated encasements. To infuse a heightened level of scientific rigor into the study, Factorial Completely Randomized Design (FCRD) framework was laid out. This encompassed a total of 12 distinct treatment regimens for each scrutinized crop, with each treatment regimen being replicated thrice. In order to facilitate the meticulous encapsulation and protection of the invaluable produce, specially crafted encasements fashioned from Low-Density Polyethylene (LDPE) and High-Density Polyethylene (HDPE) were employed. These encasements were characterized by dimensions measuring 10X12 inches and a standardized thickness of 100 microns. The treatment configurations mentioned below (as outlined in Table 1) were consistently administered across all three distinct types of green leafy vegetables subject to investigation.

Table 1. Treatment protocol for enhancing postharvest preservation of GLV:

Treatment No.	Storage condition	Gas composition (O_2: CO_2: N_2)	Packing material
T1-$R_1G_1M_1$	Ambient (35±5°C)	5%:5%:90%	LDPE
T2-$R_1G_1M_2$	Ambient (35±5°C)	5%:5%:90%	HDPE
T3-$R_1G_2M_1$	Ambient (35±5°C)	6%:5%:89%	LDPE
T4-$R_1G_2M_2$	Ambient (35±5°C)	6%:5%:89%	HDPE
T5-$R_1G_3M_1$	Ambient (35±5°C)	4%:5%:91%	LDPE
T6-$R_1G_3M_2$	Ambient (35±5°C)	4%:5%:91%	HDPE
T7-$R_2G_1M_1$	Refrigerated (5±5°C)	5%:5%:90%	LDPE
T8-$R_2G_1M_2$	Refrigerated (5±5°C)	5%:5%:90%	HDPE
T9-$R_2G_2M_1$	Refrigerated (5±5°C)	6%:5%:89%	LDPE
T10-$R_2G_2M_2$	Refrigerated (5±5°C)	6%:5%:89%	HDPE
T11-$R_2G_3M_1$	Refrigerated (5±5°C)	4%:5%:91%	LDPE
T12-$R_2G_3M_2$	Refrigerated (5±5°C)	4%:5%:91%	HDPE

Where, R – Storage condition, G – Gas composition, M – Packing substrate

2.2 Physiological loss in weight (PLW)

In order to gain insights into the shelf life of the produce, PLW measurements were recorded on a daily basis for all the packaged samples stored under both conditions. The loss in weight was calculated using a well-established formula, expressed in percentage. This approach allowed for a quantitative assessment of the weight reduction experienced by the stored produce over time, providing valuable information on its physiological changes and perishability.

$$Physiological\ Loss\ in\ Weight = \frac{Initial\ weight(g) - Final\ weight(g)}{Initial\ weight(g)}\ X100$$

2.3 Statistical analysis

The collected data was subjected to rigorous processing and analysis using the widely employed Microsoft Excel software. Furthermore, the robust statistical software, R Studio, was employed to conduct an in-depth analysis of the obtained data. Specifically, the focus of the analysis revolved around investigating the extent of PLW observed in GLV across the various treatment conditions. To ascertain the significance of observed differences, a widely accepted statistical technique, Analysis of Variance (ANOVA), was meticulously adopted to discern statistically meaningful variations among the treatment groups.

3. RESULT AND DISCUSSION

Irrespective of the gas composition and storage temperature, Low-Density Polythene packing materials performed better in holding the shelf life of produce when compared to the High-Density Polythene. This was framed based on the conclusion derived from both appearance as well as physiological loss in weight. Also, the produce stored in refrigerated had a longer shelf life than that stored under ambient condition. This could be said in aspects of days taken to start the reduction in shelf life of the produce, say ooze out from the produce and also loss in characteristic features like colour, texture and firmness of the produce. In a similar vein, a study conducted elucidated that French bean exhibited elevated PLW when subjected to storage under ambient conditions. For the preservation of vegetables, it is highly advisable to adopt a storage temperature of 5°C within a modified atmospheric package [5]. This approach has been found to effectively retain the vegetable's quality attributes even after a considerable duration of storage. Moreover, when ready-to-eat vegetables are stored at refrigeration temperatures, it serves to considerably prolong their shelf life by impeding the growth rate of microorganisms [6]. The physiological weight loss in verdant leafy vegetables is been illustrated in Table 2.

Table 2. Physiological weight loss indices in GLV under diverse treatments

Treatment	Lettuce				Palak			
T1	2.50				1.167			
T2	2.720				0.373			
T3	0.580				0.190			
T4	0.940				1.170			
T5	0.380				0.180			
T6	0.450				0.573			
T7	0.560				0.967			
T8	0.940				3.000			
T9	0.690				0.610			
T10	1.250				8.117			
T11	0.540				0.770			
T12	0.830				1.030			
	R	**G**	**M**	**RGM**	**R**	**G**	**M**	**RGM**
S. Ed	0.013	0.016	0.013	0.032	0.063	0.077	0.063	0.153
CV	0.027***	0.033***	0.027***	NS	0.129***	0.158***	0.129***	0.316***

Where, R – Storage condition, G – Gas composition, M – Packing substrate

**** significant at 0.001 level*

3.1 Longevity of Lettuce

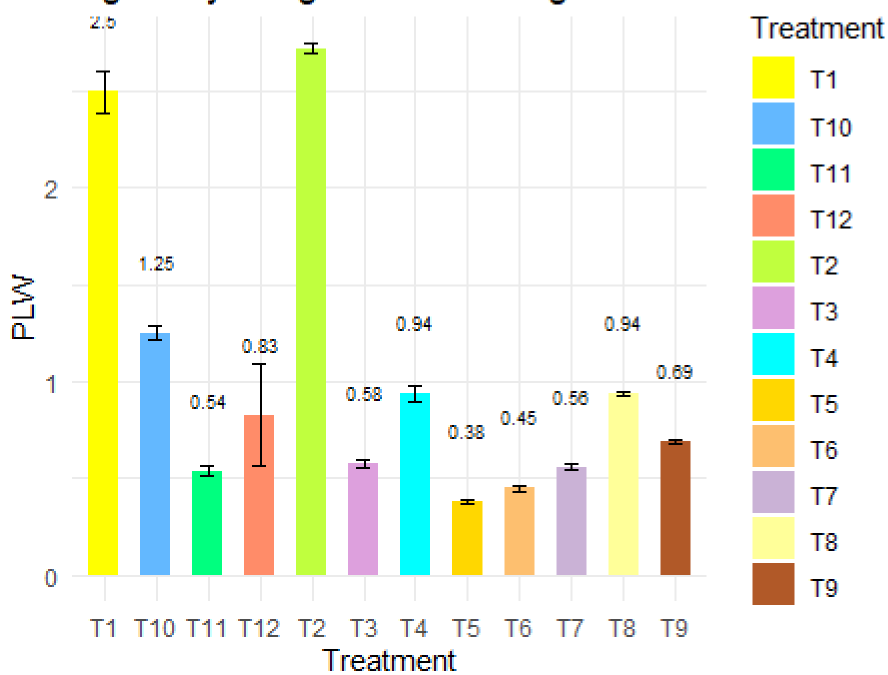
In the case of lettuce stored under ambient conditions (Fig 3 and Table 3), T5 (0.38 %) was noted to perform better followed by T6 (0.45%) and T3 (0.58 %) in case of physiological loss in weight. At the same, time the produce stored using T2 (2.72 %) and T1 (2.5 %) performed comparatively very poor from rest of the treatment sets. In the case of lettuce stored in refrigerator condition (Fig 5), T11 (0.54 %) followed by T7 (0.56 %) and T9 (0.69 %) performed better than the rest treatment. Also, the highest physiological loss in refrigerated produce, weight was noticed in produce packed using HDPE i.e., T10 (1.25%) performed in a detoriative manner. In comparison with both the storage condition refrigerated once were proven to be best on the basis of the number of days consumed, ambient stored produce hardly lasted for 5 days after which the lost the characteristic feature of lettuce which is firmness. Whereas in the case of refrigerated stored produce this loss in the characteristic feature was noticed only by 15 days after storage (DAS). In specific T11 and T7 would be recommended to be followed in the commercial manner for lettuce crop. This clear variation is pictorially and graphically been illustrated in Fig 1.

Table 3: Physiological loss undergone in various treatments from Day 1 to 15:

Treatment	DAY 1	DAY 3	DAY 5	DAY7	DAY 9	DAY 11	DAY 13	DAY 15
T1	0	0.52	1.67	-	-	-	-	-
T2	0	0.11	0.37	-	-	-	-	-
T3	0	0	0.19	-	-	-	-	-
T4	0	0.03	1.17	-	-	-	-	-
T5	0	0.18	0.18	-	-	-	-	-
T6	0	0.19	0.57	-	-	-	-	-
T7	0	0	0.19	0.58	0.63	0.76	0.85	0.97

T8	0	0	0.18	0.18	0.63	2.45	2.94	3
T9	0	0.03	0.07	0.19	0.31	0.38	0.54	0.61
T10	0	6.99	6.99	7.02	7.34	7.56	7.75	8.12
T11	0	0	0.19	0.19	0.38	0.58	0.62	0.77
T12	0	0.16	0.19	0.69	0.76	0.76	0.84	1.03

Fig 1:Physiological loss in weight indices in Lettuce



3.2 Longevity of Palak

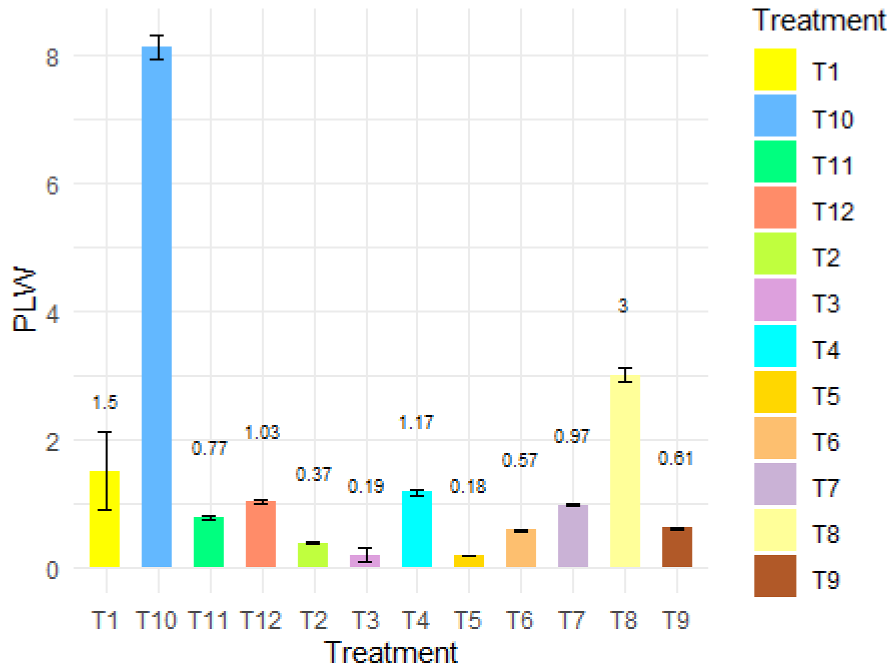
In the case of palak stored under ambient condition (Fig 4 and Table 3), T5 (0.18 %) was noted to perform better followed by T3 (0.19 %) and T2 (0.37 %) in case of physiological loss in weight. At the same time, the produce stored using T1 (1.5 %) performed comparatively very poor from the rest of the treatment sets. In the case of palak stored in refrigerator condition (Fig 6), T9 (0.61 %) followed by T12 (0.77 %) and T7 (0.97 %) performed better than the rest treatment. Also, the highest physiological loss in refrigerated produce and weight was noticed in produce packed using LDPE with first gas composition (6%:5%:89%) i.e., T10 (8.12 %) performed in a detoriative manner. In comparison with both the storage condition refrigerated once were proven to be best on the basis of the number of days consumed, ambient stored produce hardly lasted for 5 days after which the loss in the texture in the produce was noted. Whereas in the case of refrigerated stored produce this loss in the texture was noticed only by 15 days after storage (DAS). In specific T9 and T11 would be recommended to be followed in a commercial manner for palak crop. These physiological loss in weight indices under diverse treatment combination are picturized in Fig 2. The observed decline in postharvest physiological weight (PLW) of palak may plausibly be attributed to the emergence of elevated relative humidity conditions in the controlled environment, which subsequently contributed to the suppression of palak's respiration rate. In a previous study, it was found that palak packed under LDPE exhibited a notable reduction in physiological loss of weight compared to the other treatment methods [15]. Additionally, the evaluation of ethylene levels in this treatment indicated favorable outcomes by showing a detrimental effect on ethylene production.

Table 4: Physiological loss undergone in various treatments from Day 1 to 15:

Treatment	DAY 1	DAY 3	DAY 5	DAY7	DAY 9	DAY 11	DAY 13	DAY 15
T1	0	0.76	2.5	-	-	-	-	-
T2	0	0.18	2.72	-	-	-	-	-
T3	0	0.38	0.58	-	-	-	-	-

T4	0	0.75	0.94	-	-	-	-	-
T5	0	0.38	0.38	-	-	-	-	-
T6	0	0.16	0.45	-	-	-	-	-
T7	0	0	0.19	0.22	0.37	0.49	0.52	0.56
T8	0	0	0.11	0.22	0.26	0.56	0.68	0.94
T9	0	0	0	0.11	0.24	0.38	0.47	0.69
T10	0	0.26	0.37	0.56	0.56	0.94	0.98	1.25
T11	0	0	0.03	0.19	0.19	0.34	0.38	0.54
T12	0	0	0.18	0.18	0.37	0.49	0.71	0.83

Fig 2:Physiological loss in weight indices in Palak



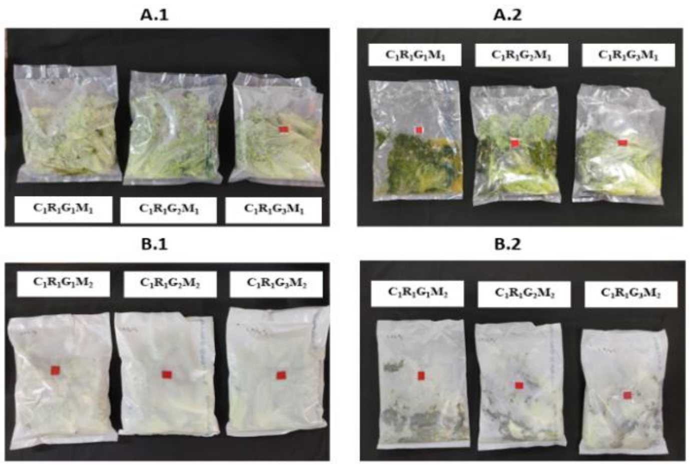


Fig 3: Lettuce stored under ambient condition: lettuce packed on day one (LDPE – A.1 and HDPE – B.1) and lettuce on 5th day of packing (LDPE – A.2 and HDPE – B.2)

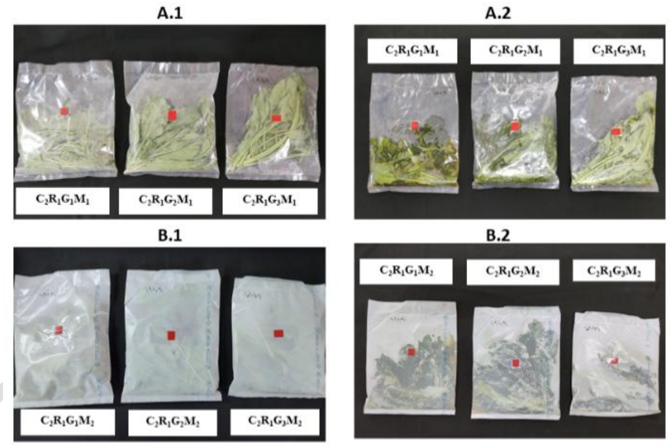


Fig 4: Palak stored under ambient condition: palak packed on day one (LDPE – A.1 and HDPE – B.1) and palak on 5th day of packing (LDPE – A.2 and HDPE – B.2)

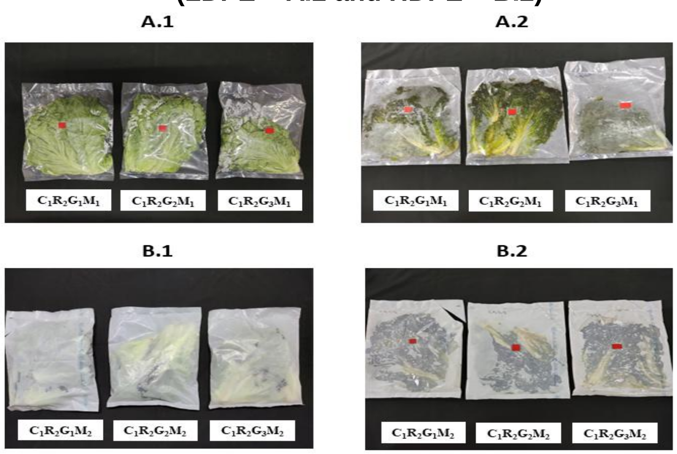


Fig 5: Lettuce stored under refrigerated condition: lettuce packed on day one (LDPE – A.1 and HDPE – B.1) and lettuce on 15th day of packing (LDPE – A.2 and HDPE – B.2)

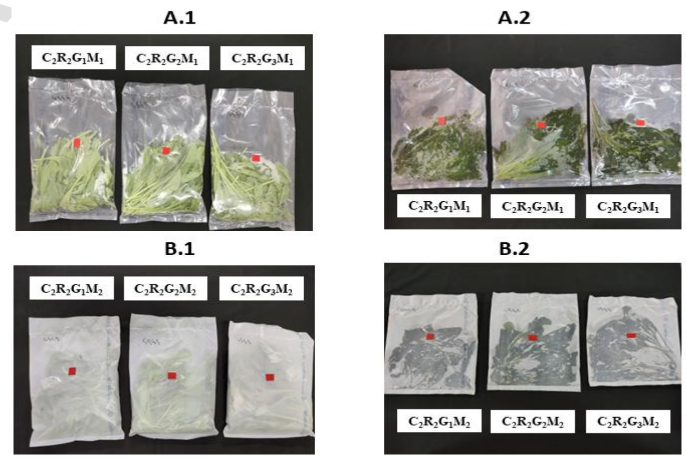


Fig 6: Palak stored under refrigerated condition: palak packed on day one (LDPE – A.1 and HDPE – B.1) and palak on 15th day of packing (LDPE – A.2 and HDPE – B.2)

The pivotal role of polyethylene in mitigating physiological loss in crop produce is well recognized. Prior research postulates that this effect could be attributed to the gas-impermeable nature of polyethylene, which curtails gaseous exchange, leading to reduced respiration and moisture loss in stored produce [7]. In the context of Amaranthus storage, a comprehensive investigation was conducted involving polyethylene bags with varying degrees of perforation, housed within a Zero Energy Cooling Chamber (ZECC) [8]. Interestingly, the nonperforated polyethylene bags emerged as the most efficacious packaging material, significantly prolonging the storage life of Amaranthus, and by extension, showcasing its potential as a viable preservation solution for Cilantro [9].

Further studies have underscored the advantages of polyethylene film packaging in conjunction with low-temperature storage. This approach has demonstrated its potential in extending the postharvest quality of *Amaranthus tricolor* leaves [10]. The refrigerated conditions (5±1℃) employed during storage yielded notable benefits, including minimized water content loss, which can be attributed to suppressed respiration and senescence-related metabolic processes [11,13]. These findings align with similar research, which revealed a direct correlation between storage temperature and weight loss in lettuce leaves, particularly when the storage duration exceeded 170 hours. The study further delineated the superiority of refrigerated storage (5±1℃) in preserving the physio-chemical attributes of leafy vegetables compared to both Zero Energy Cooling Chamber (ZECC) and ambient room temperature storage [12]. From the result it is obvious that the refrigerated produce holds its shelf life longer than that of the ambient stored produce Optimal storage conditions for modified atmosphere packaging (MAP) storage involve the implementation of low temperatures alongside specific atmospheric compositions, typically encompassing 3-8% CO_2 and 2-5% O_2 [19,20,21].

4. CONCLUSION

In culmination, this study underscores the pivotal role played by the judicious selection of packaging materials and precise storage conditions in upholding parsley's quality and prolonging its shelf life. The synergistic use of Low-Density Polyethylene (LDPE) and High-Density Packaging materials, coupled with refrigerated storage, emerges as an optimal strategy for safeguarding the freshness and extending the viability of parsley produce. These findings not only provide invaluable insights for strategic commercial practices aimed at preserving leafy vegetables but also hold the promise of enhancing their market value while curbing post-harvest losses. Furthermore, the implications of our research suggest that the adoption of Modified Atmosphere Packaging (MAP) technology, particularly the implementation of a passive atmosphere, could offer a promising avenue for retarding the deterioration of freshly chopped parsley. This approach presents significant potential, particularly within the realm of food catering establishments. Nevertheless, to fully harness the efficacy of this approach, continued refinement of the methodology is imperative. This paves the way for further exploration and optimization of this technology, ultimately contributing to the advancement of sustainable and quality-driven post-harvest practices.

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