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(RESEARCH ARTICLE)

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# Improving the post-harvest shelf-life of *Abelmoschus esculentus L.* using NOP-1 Octapeptide

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## Abstract

Increasing the post-harvest shelf life of rapidly perishable vegetables is a daunting task. In the case of *Abelmoschus esculentus L*, pod hardening and browning of the pod ridges reduces its commercial value and nutritional worth. Different methods like, use of ethylene inhibitors like 1-methylcyclopropene (1-MCP), 1-Aminoethoxyvinylglycine (AVG); salt solutions like 1% and 2% calcium chloride ($CaCl_2$) and application of synthetic peptide inhibitors are adopted to enhance the shelf life of rapidly perishable fruits, vegetables and flowers. This study aims to find out the efficacy of exogenous surface application of synthetically derived octapeptide NOP-1 (LKRYKRRL-$NH_2$) treatment at concentration of 1000µM for retarding the rapid over-ripening in *A. esculentus L*. The *A. esculentus L*. pods were stored under two conditions, at ambient room temperature & at cold storage for stipulated duration. The total flavonoid, phenolic and anti-oxidant contents were investigated. It was observed that NOP-1 peptide treatment helped to maintain the quality of the pods under both ambient and cold temperatures up-to 8 days. Anti-senescence plant derived peptide (NOP-1) adopted for study was noticed to improve the storage stability in terms of maintaining the phytochemical compositions of *A. esculentus L*. pods. The possible mechanism could be the binding of NOP-1 peptide to the ethylene receptor (ETR-1) thus reducing ethylene perception and subsequently ethylene induced ripening. The concept is new and can be safely explored for FSSAI food regulations standards. The success of this study will give higher commercial value and prolonged shelf life for short shelf-life vegetables like *Abelmoschus esculentus L.*

**Keywords:** Shelf-life; Octapeptide treatment; Post- harvest quality; Competitive inhibition; Pod over ripening

## 1. Introduction

*Abelmoschus esculentus L. Moench,* popularly known as Ladies' finger or Okra is widely consumed in the tropical and subtropical regions [1]. *A. esculentus L.* pods are known for its therapeutic properties like anti-obesity, anti-diabetic, anti-oxidant, anti-cancer, microbicidal, immunomodulatory properties and in the treatment of cardiopathy [1 and 2]. Despite its advantageous medicinal properties, *A. esculentus L.* pods have very brief shelf life and the pods rapidly over ripen post-harvest due to reduced degradation of cellulose and lignin [3].

Calcium ions treatment of fruits and vegetables is reported to help in maintaining the firmness and membrane integrity of the fruit body for extended storage. Calcium salts treatments however have been less effective when used alone and combinatory treatments like dual application of ethylene inhibitors or gamma irradiation treatments with calcium salts are reported to be effective and expensive [4].

Recently, a novel method has been employed for retarding flower senescence and delaying post-harvest fruit ripening via application of synthetic octapeptide LKRYKRRL-$NH_2$ (NOP-1) in carnation cut flowers [5], roses [6], tomatoes [7] and cut broccoli florets [8]. Studies on ethylene signaling pathways and mechanisms in model plant *Arabidopsis thaliana*

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has demonstrated that the endoplasmic reticulum (ER) integral membrane protein ETHYLENE IN-SENSITIVE 2 (EIN2) implements a positive regulatory role on ethylene signaling cascades. The EIN2 protein undergoes phosphorylation-dependent proteolytic processing and translocation of a highly conserved NLS (nuclear localization signal) at the EIN2 C-terminal into the nucleus to induce expression of ethylene responsive genes [6]. The NOP-1 peptide mimics the NLS motif of EIN2 and competitively binds to the GAF (cGMP-specific phosphodiesterase, adenylyl cyclases, and FhlA) domain of Ethylene Receptor -1 (ETR-1). This interferes and inhibits the EIN2– ETR-1 interaction to reduce ethylene perception [5 and 6]. This brings novel utility in controlling plant ethylene responses like delaying over-ripening of *A. esculentus L.* pods [5, 6, 7, 8 and 21]. The major attraction in the deployment of NOP-1 octapeptide is that it is reported to be safe, non-toxic, eco-friendly and water soluble [7, 8 and 25].

### *Objective of the study*

The purpose of the study was to evaluate the changes brought about by the surface application of NOP-1 peptide in the storage quality of *A. esculentus L.* pods. This was investigated by studying the anti-oxidant potential and phytochemical quality retention in post-harvest stored *A. esculentus L.* pods kept at room temperature (24 ± 2 ˚C) for up-to 8 days and at cold storage (6 ± 2 ˚C) for up to 20 days. The novelty of this work was in comparing the efficacy of peptide treatment under different temperatures and duration.

## 2. Material and methods

### 2.1. Plant materials and Treatment

The *A. esculentus L.* pods were purchased from local market, washed with distilled water and air dried. The synthetic plant derived octapeptide NOP-1 was customized and purchased from Priveel Peptides, Chennai. The experiment was planned to compare efficacy of peptide treatment against 1% and 2% $CaCl_2$ solution (positive control) and distilled water (negative control). The 1000 µM octapeptide solution was prepared by dissolving in distilled water and the *A. esculentus L.* pods were surface coated evenly using a fine brush. The pods were then let to air dry at room temperature and then packed in food grade polythene bags and stored for experimentation. The pods samples were segregated into two groups, one at room temperature (24 ± 2 ˚C) for up to 8 days storage and second at cold storage treatment (6 ± 2 ˚C) for up to 20 days where, 3 pods each were collected for every $4^{th}$ day as shown in Fig1.

**Figure 1** *A. esculentus L.* pods experiment set up

### 2.2. Preparation of aqueous pod extract

The pods were sliced and blended with the addition of distilled water, filtered and stored at 4 ˚C for future experiments.

### 2.3. Biological Evaluation Methods

#### *2.3.1. Total Phenolic content determination (TPC)*

The total phenolic content was estimated using the protocol adopted with slight modifications [9]. Gallic acid (GAE) standard was prepared in distilled water and different concentrations [50-1000µg/mL] were taken. Folin-Ciocalteu

reagent was used at 1:10 dilution. The plant extract & gallic acid standards taken in separate test tubes, 5ml of Folin-Ciocalteu reagent. Then 4ml of 1M $NaCO_3$ was added and incubated at room temperature for 15 minutes. The absorbance was checked at 765nm and repeated in duplicates. The concentration is expressed as µg GA/mg sample.

*2.3.2. Total Flavonoid content determination (TFC)*

To find the estimate of total flavonoid content, 500 µL of the sample was added with 100µL aluminium chloride (10%), 1500 µL methanol, 100 µL potassium acetate (1 M) and 2800 µL distilled water. After 30 min room temperature incubation, the absorbance was read at 415nm. Quercetin was used as standard (50-1000 µg/mL) and the total flavonoid content of the samples was expressed as µg QE/mg sample. The experiment was done as duplicate [10, 11].

*2.3.3. DPPH Free Radical Scavenging Assay*

The antioxidant potentials of the samples were determined by measuring the DPPH (2,2-Diphenyl-1-picrylhydrazyl) free radical scavenging activity [9 ,10 and 11]. To 20 µL of sample, 180 µL of 0.1mM DPPH solution was added and incubated at dark for 30 minutes at room temperature. The absorbance was measured at 517nm and the % inhibition was measured using formula 1. The experiment was done as duplicate.

$$\% \text{ of Scavenging} = \frac{(Pc-Ps)}{Pc} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots 1$$

Where Pc is the absorbance of control sample and Ps is the absorbance of the sample.

*2.3.4. Total Reducing Power Assay*

The reducing power assay was performed using the standard protocol [9] with slight modifications. 200 µL of sample was taken and to it 200 µL of 0.2M phosphate buffer (pH 6.6) and 250 µL of 1% potassium ferric cyanide were added and incubated at 50˚C for 20 minutes and allowed to cool. The reaction was stopped via addition of 10% tri-chloro acetic acid (TCA) and centrifuged at 3000rpm for 20 minutes. 150 µL of supernatant was carefully pooled out and 50 µL of 0.1% $FeCl_3$ solution was added and finally the absorbance was measured at 700 nm. Ascorbic acid was used as the standard and for the blank solution, phosphate buffer was used. Increase in the absorbance of the reaction mixture indicates stronger reducing power.

### 2.4. Statistical analysis

All the experiments were performed in duplicates and data analyzed in MS Excel. Results are expressed as means ± standard deviation.

## 3. Results and discussion

### 3.1. Total phenolic content determination (TPC)

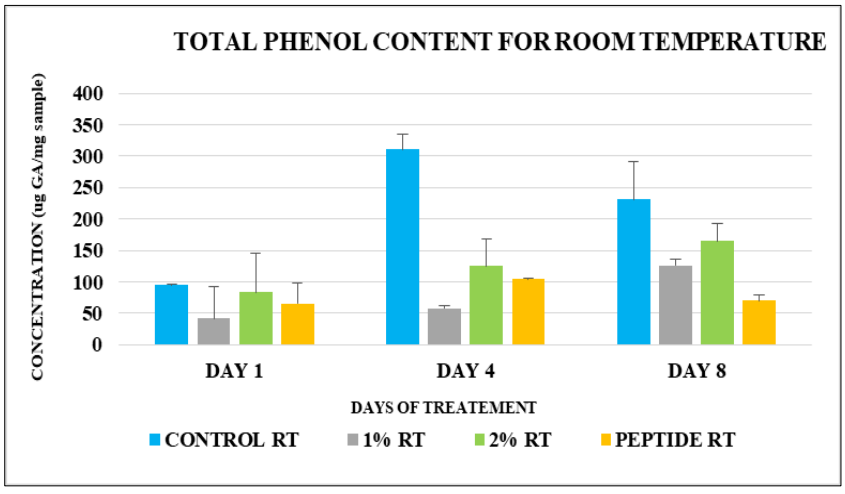


**Figure 2 (A)** *A. esculentus L.* pods Total Phenol content for room temperature storage. Each value represents the mean ± standard errors of two replications

The phenolic compounds accumulation is attributed to browning of vegetables and fruit surfaces and thus not desirable for long term storage [12, 13, 22 and 24]. It was observed that surface coating of octapeptide NOP-1 retarded the accumulation of phenolics compared to control treatments for both the test conditions as shown in Fig 2(A) & Fig 2(B).

The total phenolic content for the control, 1% and 2% $CaCl_2$ treated samples were seen to increase more than the octapeptide NOP-1 treated samples. The NOP-1 treated samples showed consistent and gradual raise at room temperature as in Fig 2(A) with the highest concentration of 103.2 ± 2 µg GA/mg sample observed for the 4$^{th}$ day. On the other hand, all the cold storage samples exhibited elevated accumulation of phenolic concentration, highest at 347 ± 40 µg GA/mg sample on the 12$^{th}$ day owing to the chilling injury experienced by the pods during prolonged storage [12] as in Fig 2(B). Similar such observations of phenolic accumulation during cold storage were also made in anthurium cut flowers with 2000 µg/ mg sample for 20$^{th}$ day treatment [14] and in ethylene treated broccoli at 1380 ± 11 µg/ mg sample of phenolic concentration during room temperature storage [8].

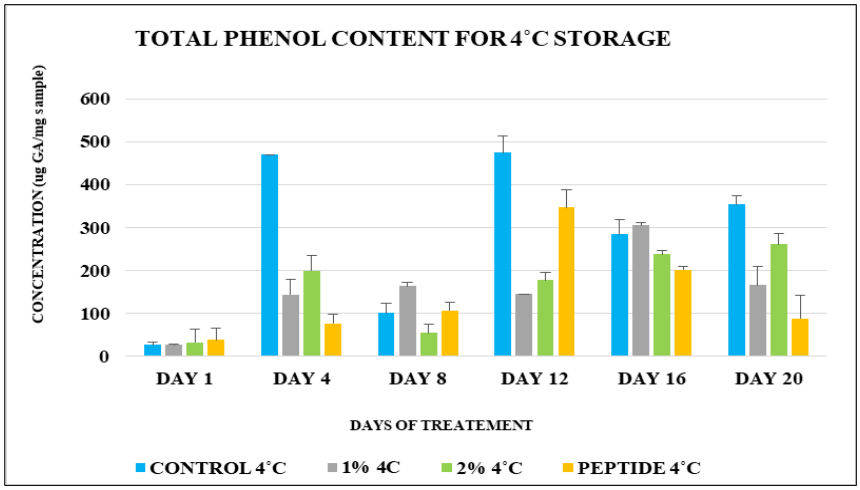


**Figure 2 (B)** *A. esculentus L.* pods Total Phenol content for 4˚C storage. Each value represents the mean ± standard errors of two replications

### 3.2. Total flavonoid content determination (TFC)

Various storage conditions and harvest times have been be recorded to impact the post-harvest flavonoid accumulation in apples, tomatoes and flowers like marigold [15]. The quantitative flavonoid estimations for room temperature and cold storage conditions are shown in Fig 3(A) & Fig 3(B) respectively.

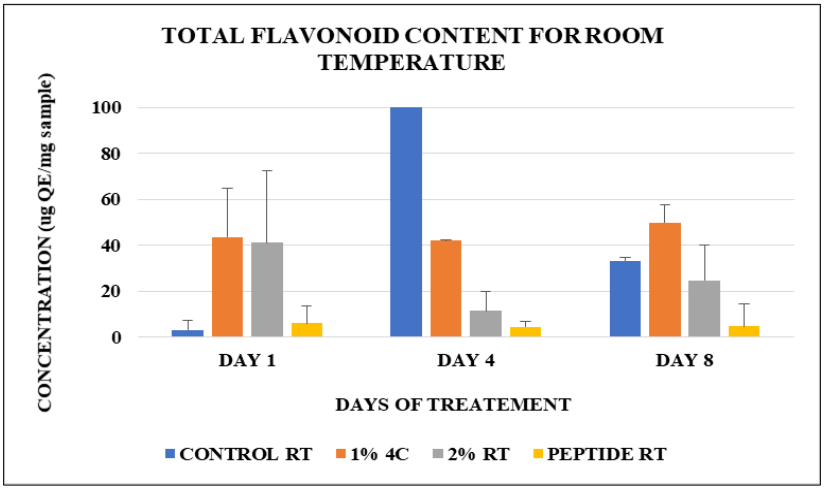


**Figure 3 (A)** *A. esculentus L.* pods Total Flavonoid Content estimation for room temperature. Each value represents the mean ± standard errors of two replications

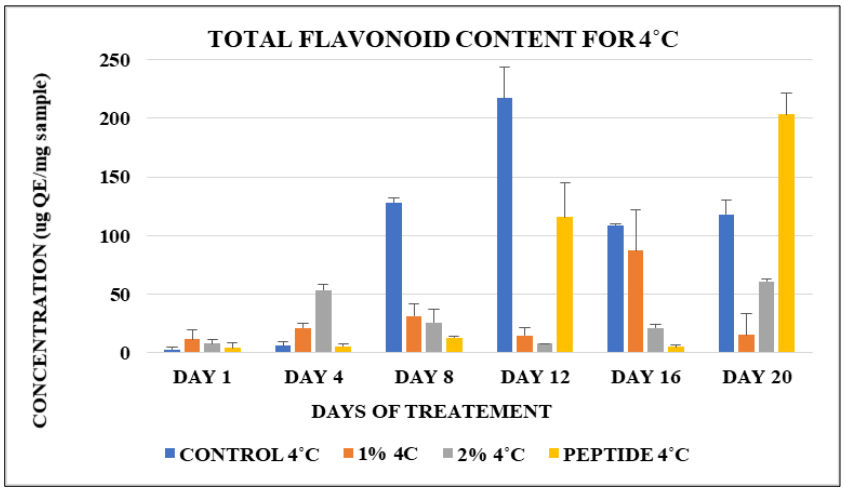


**Figure 3 (B)** *A. esculentus L.* pods Total Flavonoid Content estimation for 4˚C. Each value represents the mean ± standard errors of two replications

It was observed that under ambient room temperature, the NOP-1 octa-peptide treated *A. esculentus L.* fruit pods had flavonoid content consistent around 5 ± 0.5 µg QE/mg sample without much variations up-to 8 days as in Fig 3(A). The NOP-1 treated cold storage samples however had elevated flavonoid accumulation after 8$^{th}$ day and the highest recorded on day 12 and on day 20 as 116 ± 30 µg QE/mg sample and 203.33 ± 18 µg QE/mg sample respectively. There was a compromise on the fruit pod weight and degradation with prolonged storage as in Fig 3(B). Increase in flavonoid concentration is relatable to activation of various flavonoid bio-synthesis pathways and consequent abiotic stress induced response [15 and 16]. Similar such elevated flavonoids levels upon post-harvest treatment via elevated expression of flavonoid bio-synthesis genes were reported in ginkgo leaves [16] & in litchi fruits [17].

### 3.3. DPPH Free Radical Scavenging Assay

DPPH is an easy, quick and sensitive assay adopted often to qualitatively determine the antioxidant activity of plant extracts [10 and 11]. The DPPH anti-oxidant inhibitory activity is known to have a positive correlation with the availability of phenolic derivatives [2,9, 22 and 24]. It was noticed that the radical scavenging activity was consistent for all the treatments and conditions, at room temperature as shown in Fig 4(A) & at 6 ˚C as in Fig 4(B).

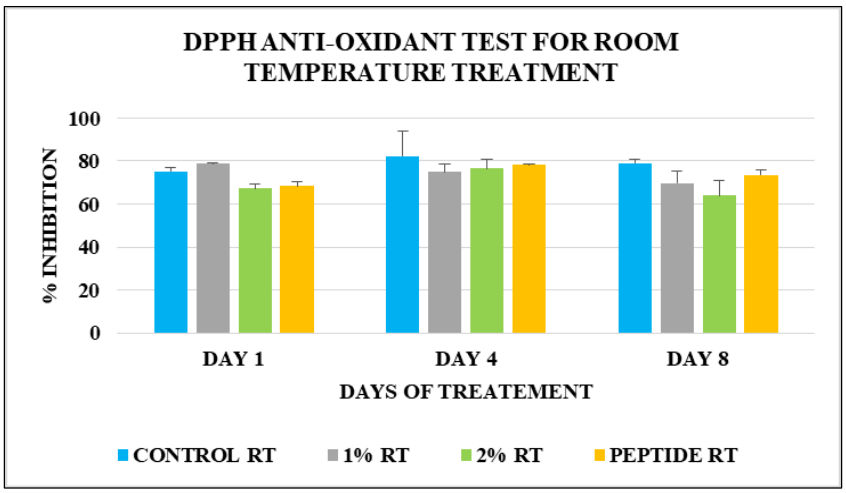


**Figure 4 (A)** *DPPH* Anti-Oxidant Test for room temperature. Each value represents the mean ± standard errors of two replications

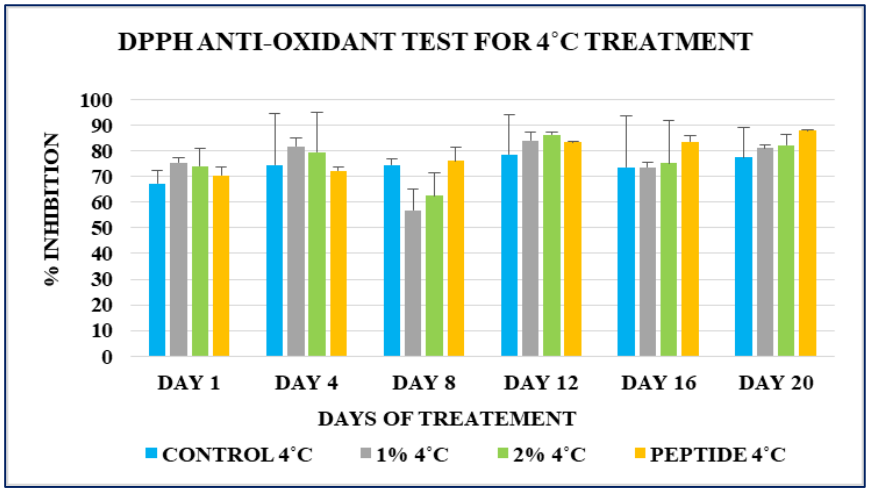


**Figure 4 (B)** *DPPH* Anti-Oxidant Test for 4˚C treatment. Each value represents the mean ± standard errors of two replications

The *A. esculentus L.* fruit pods stored at ambient temperature was noticed to have approximately 73 ± 4.85 % inhibitory activity up-to 8 days. The NOP-1 treated cold storage samples however were noticed to have higher inhibition beyond 8 days which qualitatively helps to confirm the presence of phenolic and flavonoid compounds accumulation due to prolonged storage as found in ginkgo leaves [16 and 18].

### 3.4. Total Reducing Power Assay

The total reducing potential of plant extracts attributes to the anti-oxidant activity of plant phytochemicals [18 and 19]. *A. esculentus L.* pods are reported to be a rich source of phenolic compounds like catechin, iso-quercitrin and rutin [2]. The total reducing power of treated *A. esculentus L.* pods for room temperature and cold storage conditions are shown in Fig 5(A) & Fig 5(B) respectively.

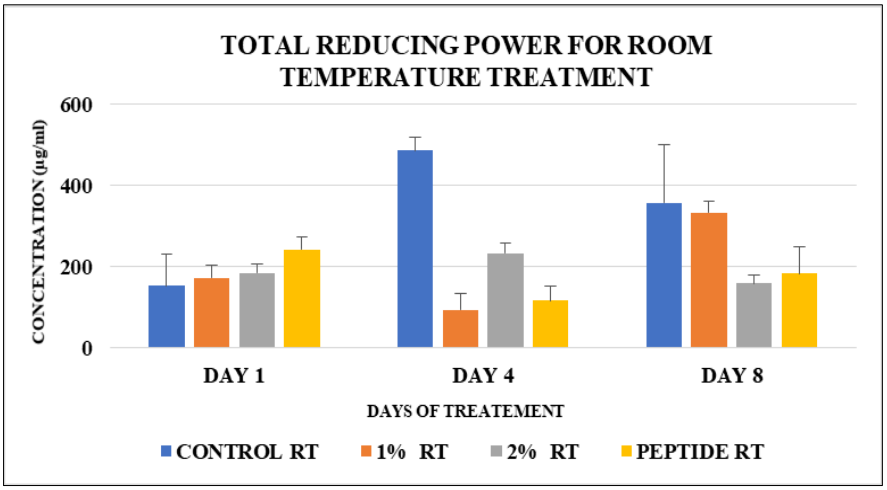


**Figure 5 (A)** Total Reducing Power for *A. esculentus L.* pods at room temperature treatment. Each value represents the mean ± standard errors of two replications

The reducing potential of NOP-1 octapeptide treated samples at room temperature and cold storage were seen to be 183 ± 64 µg/ml and 79.83 ± 78.1 µg/ml respectively for day 8. The increased reducing power activity in the cold stored *A. esculentus L.* pods is associable with the observed elevated level of phenolics and flavonoids in correspondence to prolonged storage [12, 20, 21, 22, 23, 24 and 25]. However, the fruit pods had observable moisture and weight loss beyond 8 days under both temperature conditions.

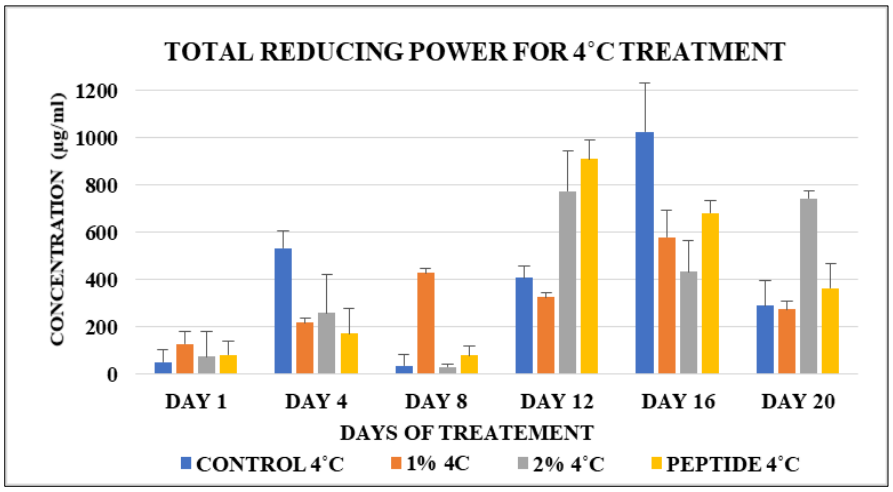


**Figure 5 (B)** Total Reducing Power for *A. esculentus L.* pods at 4˚C. Each value represents the mean ± standard errors of two replications

## 4. Conclusion

Post-harvest browning and weight loss of stored *A. esculentus L.* pods are one of the most crucial factors limiting its commercial and nutritional value. In the current study, the anti- senescence activity of synthetic NOP-1 peptide on *A. esculentus L* pods for delaying the post-harvest aging was observed. The NOP-1 octapeptide treatment helped in maintaining the quality, phytochemical compositions of total flavonoid and phenolic contents in *A. esculentus L* pods up to 8 days under both ambient and cold storage conditions and beyond which the fruit pods lost its freshness and moisture. The total phenolic test, DPPH test and reducing power assay revealed that NOP-1 peptide treatment helped to maintain the total phenolic profile of the *A. esculentus L* pods under both conditions compared to the control and calcium chloride treatments. The enhanced total flavonoid concentration found in cold and room temperature stored pods is associable to abiotic stress experienced by the pods due to prolonged storage after 8 days.

NOP-1 peptide treatment can be considered as a promising, safe, and eco-friendly treatment strategy which is easy to dissolve in water and can be used readily for various horticulture purposes to keep vegetables, flowers and fruits fresh for prolonged storage. The future work can be extended to examine the profile of crucial molecular and biochemical markers / genetic hallmarks like autocatalytic ethylene biosynthesis genes and stress inducible plant hormone genes which are found to be over-expressed upon ethylene induced over-ripening conditions.

## Compliance with ethical standards

### *Acknowledgments*

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### *Disclosure of conflict of interest*

The Authors of this work declare no conflict of interests.

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