1
Annals
Agric. Sci.,
Ain Shams Univ.,
Cairo, 52(2), 367-374, 2007

EVALUATION OF FREE RADICAL SCAVENGING BY NATURAL ANTIOXIDANTS IN SOME FRESH, BLANCHED, COLD AND FROZEN STORAGE OF VEGETABLES

[29]

Abd EL-Malak[1], G.A.

1. Department of Horticultural Crops Technology Research , Food Technology Research Institute, Agricultural Research Center, Giza, Egypt

Keywords: Radical scavenging activities, Antioxidants, Blanching, Storage, Vegetables

ABSTRACT

Different pretreatments before freezing and frozen storage for 3 and 6 months of some vegetables namely jew's mallow, green beans and okra as well as cold storage at 5±1°C and 90-95%RH were investigated pertaining the effect of these treatments on natural antioxidants as free radical scavenging. Results showed that the total phenolics and flavonoids were increased after cold storage at5±1°C for 8 days while ascorbic acid and carotenoids contents were decreased. A slight decrement was observed in both total phenolics and flavonoids as a result of blanching by steam for 5min comparing to blanching in boiling water for 3.5min. At the same time the percents of ascorbic acid and total carotenoids were higher with steam blanching comparing with blanching in boiling water. Extraction of all processed samples showed different decreases in all natural antioxidants after frozen storage for 3 and 6 months. Results also revealed that the highest free radical reduction showed with cold storage at5±1°C and 90-95% RH while blanching by boiling water gave the lowest free radical reduction.

(Received June 19, 2007)
(Accepted August 13, 2007)

INTRODUTION

The effect of pretreatments of vegetables on the components for either preservation or storage was studied by many investigators. Some of these components are concerning with natural antioxidants such as polyphenols, flavonoids and carotenoids which are loss during different processing correlated by different pretreatment techniques. These compounds are affected on the free radicals which can cause damage to cellular bio-molecules such as nucleic acids, enzymes, proteins, lipids and carbohydrates and consequently may adversely affected to immune functions (**Nilsson *et al* 2004**). Recently, a number of studies on the health benefits associated with natural compounds have been demonstrated including phenolics in fruits, vegetables, herbs and spices possess effective antioxidant, anti-inflammatory, antimutagenic and anticarcinogenic activities (**Jayaprakasha *et al* 2007**). On the other hand **Rangkadilok *et al* (2007)** reported the protective effects of fruits and vegetables against chronic diseases have been attributed to the antioxidant properties of some secondary metabolites present in these foods. Also, plant polyphenols have been reported to exhibit bioactive properties and in particular antioxidant activities. The content of polyphenols as antioxidant activities and the intake quantities of polyphenols are estimated by **Thu *et al* (2004). Hollman & Katan (1999) and Nijveldt *et al* (2001)** declared that the polyphenols are abundant antioxidant in our diets and may protect the tissues in the body against oxidative stress and decrease the risk of cardiovascular diseases. Many flavonoids are found to be strong free radical scavengers and antioxidant **(Robak & Gryglewski 1988).** Moreover Carotenoids are the importance nutritional antioxidants which have been recognized possible role in the prevention of human diseases. During normal processing including blanching or storage of vegetables usually caused to distinct loss in these components i.e carotenoids, resulting in a significant quality decrease **(Ramesh *et al* 1999).**

The aim of this investigation was to studying the highest free radical scavenging activities of fresh vegetables such as jew's mallow, okra and green beans comparing with different blanching methods and storage conditions. Moreover, the effect of blanching by either dipping in boiling water or exposure to steam under normal pressure as well as cold and frozen storage for 3 and 6 months after being blanched by steam on the content of natural antioxidant activities such as polyphenols, flavonoids, ascorbic acid and carotenoids were studied.

MATERIALS AND METHODS

The various vegetables namely jew's mallow *(Corchorus olitorius L.),* okra *(Hibiscus esulentus.L).*and green beans *(Phaseolus vulgaris L.)* were obtained from local markets.

Okra and green beans were prepared by cutting to pieces ~1cm whereas Jew's mallow was finely chopped by kitchen machine. Blanching of these samples were achieved by two methods as follows: 100 grams of these samples were blanched by boiling water for 3.5min., while other 100 grams was blanched by steam under normal pressure for 5min., these periods of exposure were found to be enough to inactivate the oxidative enzymes **(Wasef *et al* 1994).** The blanched samples were cold and packaged in polyethylene bags before frozen and stored at −18°C then drawn periodically to be chemically analyzed after 3 and 6 months periods. Other fresh samples were preserved in refrigerator at 5±1°C and 90-95% RH for 8 days before analysis and fresh samples were analyzed as control.

2,2-diphenyl-1picrylhydrazyl (DPPH) was purchased from Sigma Co. and used for determination of total antioxidant activity.

Total phenolics were estimated by using photometric method with folin's reagent as reported by **(AOAC 1970).** Flavonoids were extracted and determined according to **Zhuang *et al* (1992).** While carotenoids and ascorbic acid were determined according to **(AOAC 1990).**

Total antioxidant activity

Total antioxidant activity was determined according to the method of **Brand-Williams *et al* (1995)** and modified by **Zhang & Hamauzu (2004)** as follows:

Ten grams of fresh or processed of jew's mallow, okra and green beans were homogenized with 15 mL of 80% methanol. The homogenate was filtered through four layers of cheesecloth and the residue was treated by adding with 15 mL of 80% methanol for two successive extractions. The filtrates were combined and centrifuged at 4000xg for 10 min. The supernatant of the methanol extract was collected and diluted to various concentrations (1.0%, 2.5%, 5.0%, 7.5% and 10.0%) for measuring the total antioxidant activity. The various concentration samples were studied, the concentration representing 2.5% of original fresh weight of Jew's mallow was chosen as an appropriate concentration for assessing antioxidant activity while okra and green beans the appropriate concentration were 5% in all fresh or cooked samples. Antioxidant activity was determined by the 2,2-diphenyl-1picrylhydrazyl (DPPH). An 0.1mM solution of DPPH in methanol was prepared and 4 mL of this solution were treated with 0.2 mL of the dilute extract. A control was treated with 0.2 mL of distilled water instead of the extract. The mixture was left to stand at room temperature for 60 min. before the decrease in absorbance at 517 nm was measured. Total antioxidant activity was expressed as the percentage decrease of DPPH using the equation:

$$\text{Total antioxidant activity} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

RESULTS AND DISCUSSION

Results in **Table (1)** show that the values of total phenolics content determined as tannic acid were 160.61, 62.34 and 108.80 mg/100g for fresh Jew's mallow, green beans and okra on fresh weight basis, respectively. While these amounts were increased due to cold storage up to 8 days at 5±1°C. The corresponding increment values were 33.94, 21.94 and 30.96% for Jew's mallow, green

Table 1. Effect of different treatments and storage conditions on total phenolics content (mg/100g on fresh weight basis) in jew's mallow, green beans and okra

Treatments and storage conditions	Jew's mallow	Green beans	Okra
Fresh vegetables	160.61±3.28	62.34±1.53	108.80±0.93
Steam blanching (5min)	148.18±2.90	59.68±2.98	97.46±2.81
Blanching in boiling water(3.5 min)	82.90±2.65	42.88±1.32	47.86±0.71
Cold storage*	214.91±3.82	76.04±0.70	142.48±4.02
Frozen storage for 3 months	139.82±2.13	55.47±2.16	91.53±2.11
Frozen storage for 6 months	135.27±1.72	53.57±3.38	82.38±2.57

Data are means of three replicate experiments ± SD.
* At 5±1°C and 90-95% RH for 8 day

beans and okra respectively these results are in agreement with **Leja *et al* (2001)** who stated that at the low-temperature treatment of artichoke caused the rise of phenolic components after 7 days of storage for non-packaged heads. The leaching of such components in boiling water at higher temperature compared with steam under normal pressure caused the loss could be a likely explanation for the higher percentage of phenolics by exposed to steam comparing with dipping in boiling water. The decrement percentages with steam blanched were 7.34, 4.27 and 10.42% while the phenolics after being blanched by boiling water were 48.38, 31.22 and 56.01% for Jew's mallow, green beans and okra, respectively. On the other hand, there were some decrements of phenolics were observed after 3 and 6 months of frozen storage periods at –18°C for steam blanched Jew's mallow, green beans and okra. Meanwhile, the decrement percentages after 3 months of frozen storage were 5.64, 7.05 and 6.08%, respectively, while after 6 months corresponding decrements were 8.71, 10.24 and 15.47%.

The flavonoid contents are shown in **Table (2)** as an important source of natural antioxidant, in fresh Jew's mallow, green beans and okra and after different treatments and storage conditions. The results reveal that the flavonoid contents were 107.84, 58.29 and 78.75 mg/100g in Jew's mallow, green beans and okra, respectively. Loss of flavonoids content was observed after different treatments or frozen storage except cold storage samples. These losses were different according to treatment operation prior to storage and the conditions of its storage. The flavonoid content ranged between 67.15 and 118.22 for blanched and cold storage of Jew's mallow, 27.43 and 60.94 for green beans and 35.43 and 93.12 mg/100g for okra. A moderate increase in flavonoid was observed in cold storage comparing with fresh vegetables. On the other side the lowest flavonoid content was observed with blanching processed in boiling water. The decremental percentages of flavonoids after steam blanching and frozen storage for 6 months were 13.88, 15.63 and 8.27% in Jew's mallow, green beans and okra, respectively. These decreases may be due to heat treatments of tested samples. In general both polyphenols and flavonoids act as antioxidants and free radical scavengers **(Bors & Saran 1987)**.

Table 2. Effect of different treatments and storage conditions on flavonoids content (mg /100g on fresh weight basis) in jew's mallow, green beans and okra

Treatments and storage conditions	Jew's mallow	Green beans	Okra
Fresh vegetables	107.84±1.21	58.29±1.36	78.75±2.40
Steam blanching (5 min)	104.99±3.36	53.90±2.33	73.29±3.25
Blanching in boiling water (3.5 min)	67.15±0.33	27.43±0.26	35.43±1.06
Cold storage*	118.22±1.35	60.94±2.34	93.12±1.26
Frozen storage for 3 months	98.76±3.20	52.37±2.19	70.55±1.04
Frozen storage for 6 months	92.87±2.37	49.18±1.96	67.23±2.67

Data are mans of three replicate experiments ± SD.
* At 5±1°C and 90-95% RH for 8 day

Ascorbic acid contents in fresh, blanched, cold and frozen storage of Jew's mallow, green beans and okra are shown in **Table (3)**. The highest content of ascorbic acid was observed in fresh jew's mallow while the lowest content showed in green beans. Blanching by steam caused to decompose of ascorbic acid content to 58.33, 37.52 and 49.81% in jew's mallow, green beans and okra while these decomposes due to blanching by boiling water were 94.05, 62.47 and 73.35%, respectively. After cold storage losses of ascorbic acid were observed in all samples the loss percents were 61.75, 25.35 and 10.91% for jew's mallow, green beans and okra. Frozen storage up to 3 months lead to decrease in ascorbic acid to 20.19, 21.93 and 12.56% while the decrease percents were 26.18, 34.32 and 23.90% after 6 months of frozen storage, respectively. These results are in accordance with findings by **Giannakourou & Taoukis (2003)** who reported that after the freezing/blanching process, during the subsequent isothermal frozen storage, green peas and leafy spinach exhibited a first loss of vitamin C at all temperatures studied (–3 to –20°C).

Table 3. Effect of different treatments and storage conditions on ascorbic acid content (mg/100g on fresh weight basis) in jew's mallow, green beans and okra

Treatments and storage conditions	Jew's mallow	Green beans	Okra
Fresh vegetables	158.66±2.21	15.11±0.36	28.33±0.40
Steam blanching (5 min)	66.11±1.36	9.44±0.33	14.22±0.25
Blanching in boiling water (3.5 min)	9.44±0.33	5.67±0.26	7.55±0.06
Cold storage*	60.69±0.35	11.28±0.30	25.24±0.26
Frozen storage for 3 months	52.76±3.28	7.37±0.19	11.56±0.06
Frozen storage for 6 months	48.80±0.37	6.20±0.93	10.06±0.93

Data are means of three replicate experiments ± SD.
* At 5±1°C and 90-95% RH for 8 day

Total carotenoids in items of vegetables under investigation are presented in **Table (4).** Total carotenoids were 764.59, 67.64 and 42.63 µg / 100g on fresh weight basis in fresh jew's mallow, green beans and okra, respectively. The decrement of caroteneoids in jew's mallow after steam blanching was 13.77% while for both green beans and okra were 3.78 and 4.08%. Blanching in boiling water for 3.5 min. caused to decrease the percentages of total carotenoids to 30.87, 17.77 and 24.94% for Jew's mallow, green beans and okra, respectively. The corresponding decrement values after cold storage for 8 days of total carotenoids were 37.62, 42.20 and 21.35% while percent decrements after 3 and 6 months frozen storage were 24.59 and 34.80% for jew's mallow, 19.85 and 23.48% for green beans and 20.79 and 24.97% for okra.

Table 4. Effect of different treatments and storage conditions on carotenoids content (µg/100g on fresh weight basis) in jew's mallow, green beans and okra

Treatments and storage conditions	Jew's mallow	Green beans	Okra
Fresh vegetables	764.59±4.15	67.64±2.21	42.63±1.66
Steam blanching (5min)	661.01±2.90	65.08±2.98	40.89±2.05
Blanching in boiling water (3.5 min)	528.56±2.65	55.62±1.62	32.00±0.71
Cold storage*	476.92±2.90	39.22±0.70	33.53±1.02
Frozen storage for 3 months	498.50±5.28	52.16±3.03	32.39±1.53
Frozen storage for 6 months	430.97±4.22	49.86±2.34	30.68±0.73

Data are means of three replicate experiments ± SD.
* At 5±1°C and 90-95% RH for 8 day

The method of scavenging stable DPPH free radicals can be used to evaluate the antioxidant activity of fresh, processing and storage samples extracts in a short time **(Qian & Nihorimbere 2004)**. Results in **Table (5)** show that the fresh vegetables exhibited different DPPH scavenging activities results showed that Jew's mallow had the highest activity for reducing free radicals comparing with other tested samples. The percent of antioxidant activity was 73.67% in fresh sample meanwhile some increase was observed after cold storage and blanching by steam, since percent of activities were 77.36% and 74.10%, respectively. The lowest activity of free radical scavenging showed after blanching in boiling water which was 48.64%. At the same time antioxidant activities of fresh okra and green beans were 71.38 and

21.32% while cold storage for 8 days caused an increase of DPPH scavenging activities to 81.82 and 30.68%, respectively. On the other hand, degradation values in the antioxidant activities were observed in all treatments after 3 and 6 months of frozen storage. The radical scavenging activity of the samples is due to flavonoids, phenolics acid and their derivatives **(Anagnostopoulou *et al* 2006)**.

Table 5. Effect of different treatments and storage conditions on total_antioxidant activities (%) in jew's mallow, green beans and okra

Treatments and storage conditions	Jew's mallow**	Green beans***	Okra***
Fresh vegetables	73.67±2.44	71.38±3.17	21.32±1.03
Steam blanching (5min)	74.10±2.70	68.44±1.85	25.03±2.35
Blanching in boiling water (3.5 min)	48.64±1.75	35.80±0.62	11.64±0.81
Cold storage*	77.36±2.48	81.82±2:72	30.68±1.14
Frozen storage for 3 months	62.24±3.05	63.00±1.11	17.08±0.37
Frozen storage for 6 months	59.73±2.67	48.63±0.49	11.31±0.62

Data are means of three replicate experiments ± SD.
* At 5±1°C and 90-95% RH for 8 day
** 2.5g fresh vegetables/100ml methanol
*** 5g fresh vegetables/100ml methanol

The results in **Figs. 1, 2 and 3** show that the decrease in absorbance of DPPH· radical was due to its reduction by different antioxidants. Decrease in absorbance as a result of a color change from purple to yellow as the radical was scavenged by antioxidant through donation of hydrogen to form the stable DPPH-H. Polyphenols and flavonoids were increased with cold storage process for 8 days. At the same time carotenoids and ascorbic acid were decreased. These variations reflected the inactivation and inhibition according to natural antioxidants as affected by different processes. Results in Figs. 1, 2 and 3 revealed that the highest free radical reduction was showed with cold storage while blanching by boiling water gave the lowest free radical reduction. These results may be due to keeping the large amounts of natural antioxidants. Moreover some activation was observed in okra with steam blanching treatment comparing to fresh samples. The act of natural antioxidants in fresh Jew's mallow gave the same results comparing with blanching by steam. On the other hand, some losses were observed after frozen storage for 3 and 6 months. The absorbance decrements of DPPH solution depending on the speed of the reaction between DPPH· and natural antioxidants. In case of rapid kinetic behavior, practically all samples at high concentrations were reacted within a very short time, and a steady state was reached almost immediately. On the other hand, slow kinetic behavior(lowest concentration for each sample) implied longer periods before the steady state could de reached **(Qian & Nihorimbere 2004)**. Therefore, blanched jew's mallow by steam showed rapid kinetic behavior and strong reducer of a DPPH· radical. Therefore it could be considered the fresh, cold, and frozen storage after blanched by steam of jew's mallow, green beans and okra have high antioxidant activity.

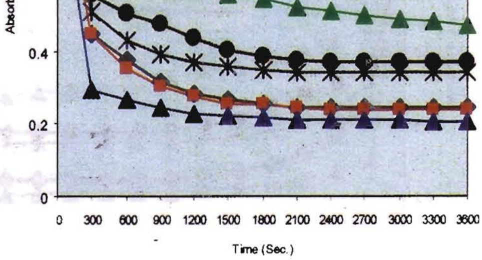

Fig. 1. Effect of different treatments and storage conditions on natural antioxidant activity as reducing DPPH for jew's mallow extracted by methanol (2.5g/100ml on FWB)

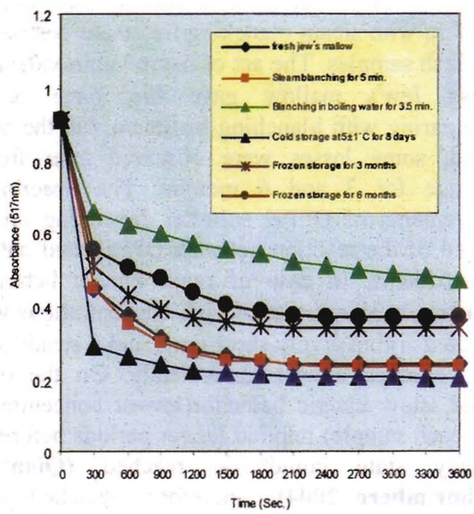

Fig. 2. Effect of different treatments and storage conditions on natural antioxidant activity as reducing DPPH for green beans extracted by methanol (5g/100ml on FWB)

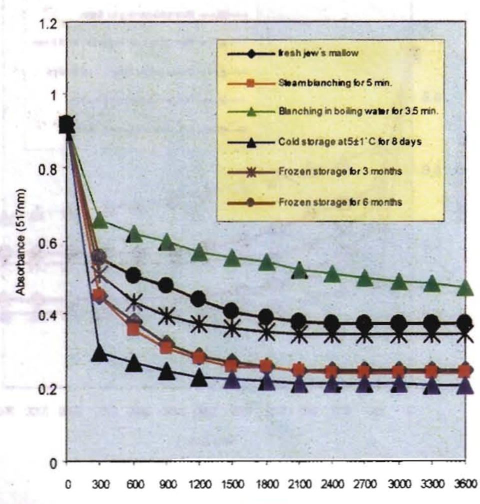

Fig. 3. Effect of different treatments and storage conditions on natural antioxidant activity as reducing DPPH for okra extracted by methanol (5g/100ml on FWB)

REFERENCES

Anagnostopoulou, M.A.; P. Kefalas; V.P. Papageorgiou; A.N. Assimopoulou and D. Boskou (2006). Radical scavenging activity of various extracts and fractions of sweet orange peel (*Citrus sinensis*). **Food Chem., 94: 19-25.**

AOAC (1970). Official Methods of Analysis pp.832-832. Association Official Analytical Chemists, Washington, DC, USA.

AOAC (1990). Official Methods of Analysis pp.1058-1061. Association Official Analytical Chemists, Washington, DC, USA.

Bors, W. and M. Saran (1987). Radical scavenging by flavonoid antioxidants. **Free Radical Res. Commun 2: 4-6.**

Brand-Williams, W.; M.E. Cuvelier and C. Berset (1995). Use of free radical method to evaluate antioxidant activity. **Lebensmittel-Wissenschaft und Technologie, 28: 25-30.**

Giannakourou, M.C. and P.S. Taoukis (2003). Kinetic modeling of vitamin C loss in frozen green vegetables under storage conditions. **Food Chem., 83: 33-41.**

Hollman, P.C. and M.B. Katan (1999). Dietary flavonoids intake, health effects and bioavailability. **Food Chem Toxical., 37: 937-942.**

Jayaprakasha, G.K.; P.S. Negi; B.S. Jena and L. Jagan Mohan Rao (2007). Antioxidant and antimutagenic activities of Cinnamomum zeylanicum fruit extracts. **Journal of Food Composition and Analysis, 20(3-4): 330-336.**

Leja, M.; A. Mareczek ; A. Starzynska and S. Rozek (2001). Antioxidant ability of broccoli flower buds during short-term storage **Food Chem., 72(2): 219-222.**

Nijveldt, R.J.; E. Nood; D.E.C. Hoorn; P.G. Boelens; K. Norren and P.A.M. Leeuwen. (2001). Flavonoids: a review of probable mechanisms of action and potential application. **Am. J. Clin. Nutr., 74: 937-942.**

Niisson J.; R. Stegmark and B. Akesson (2004). Total antioxidant capacity in different pea (*pisum sativum*) varieties after blanching and freezing. **Food Chem., 86:501-507.**

Qian, H. and V. Nihorimbere (2004). Antioxidant power of phytochemicals from *Psidium guajava* leaf. **J. Zhejiang Univ. Sci., 5 (6): 676-683.**

Ramesh, M.N.; W. Wolf; D. Tevini and G. Jung (1999). Studies on inert gas processing of vegetables. **Journal of Food Engineering, 40(3): 199-205.**

Rangkadilok, N.; S. Sitthimonchai; L. Worasuttayangkurn; C. Mahidol; M. Ruchirawat

and J. Satayavivad (2007). Evaluation of free radical scavenging and antityrosinase activities of standardized longan fruit extract. **Food Chem. Toxicol., 45(2): 328 -336.**

Roback, J. and R.J. Gryglewski (1988). Flavonoids are scavengers of superoxide anions. **Biochem. Phrmacol., 37: 837-841.**

Thu, N.N.; C. Sakurai; H. Uto; N.V. Chuyen; D.T.K. Lien; S. Yamamoto; R. Ohmori and K. Kondo (2004). The polyphenol content and antioxidant activities of the main edible vegetables in northern Vietnam. **J. Nutr. Sci. Vitaminol., 50: 203-210.**

Wasef, R.A.; H.N. Habashy and G.A. El-Sherbeiny (1994). Effect of blanching conditions on peroxidase activities and pigments in leafy vegetables. **Egypt. J. Appl. Sci., 9(5): 502-519.**

Zhang, D. and Y. Hamauzu (2004). Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking. **Food Chem., 88: 503–509.**

Zhuang, X.P.; Y.Y. Lu and G.S. Yang (1992). Extraction and determination of flavonoid in ginkgo. **Chinese Herbal Medicine, 23: 122-124.**

374
حوليات العلوم الزراعية
جامعة عين شمس، القاهرة
مجلد(٥٢)، عدد (٢)، ٣٦٧-٣٧٤، ٢٠٠٧

تقييم الشقوق الحرة المزالة بواسطة مضادات الأكسدة في بعض الخضروات الطازجة والمحفوظة بالتبريد و التجميد

[٢٩]

چورچ عبيد عبد الملاك[١]

١. قسم بحوث تكنولوجيا الحاصلات البستانية – معهد بحوث تكنولوجيا الأغذية – مركز البحوث الزراعية – الجيزة – مصر

تم دراسة استخدام التخزين بالتبريد لبعض الخضروات مثل الملوخية والبامية والفاصوليا لمدة ٨ أيام علي درجة ٥±١°م ورطوبة نسبية ٩٠- ٩٥ %. كذلك إجراء معاملات السلق بالبخار والماء الساخن ثم التعبئة والتجميد والتخزين علي – ١٨°م لمدة ٦ شهور وتأثير ذلك علي مضادات الأكسدة الطبيعية ونشاطها في التخلص من الشقوق الحرة. أوضحت النتائج أن كلا من الفينولات الكلية والفلافونات تزداد بعد التخزين بالتبريد لمدة ٨ أيام علي درجة حرارة ٥±١°م بينما لوحظ انخفاض في محتوي كل من الكاروتينات وحامض الأسكوربيك.ومن جهة أخري لوحظ أيضا حدوث انخفاض طفيف في كل من الفينولات الكلية والفلافونات بعد السلق ببخار الماء لمدة ٥ دقائق وذلك بالمقارنة بالسلق في الماء المغلي لمدة ٣,٥ دقيقة وفي نفس الوقت كانت نسبة الكاروتينات وحامض الأسكوربيك اعلي في حالة السلق ببخار الماء. هذا وقد أظهرت النتائج أيضا حدوث انخفاض بمعدلات متباينة لمضادات الأكسدة الطبيعية عند تحليل العينات المخزنة بالتجميد بعد ثلاثة و ستة شهور. أوضحت الدراسة أيضا تحول لون مادة ٢,٢- داي فينيل -١ بيكريل هيدرازيل (DPPH) التي تعمل عمل الشقوق الحرة من اللون البنفسجي إلي اللون الأصفر والتي تعكس مدي تأثير المعاملات الأولية وطرق التخزين في الحفاظ علي مضادات الأكسدة الطبيعية والتخلص من الشوارد أو الشقوق الحرة التي تنفرد أثناء العمليات الحيوية. حيث بينت النتائج أن اعلي معدل لاختزال الشقوق الحرة أمكن ملاحظته في العينات المخزنة بالتبريد لمدة ٨ أيام واقلها تأثيرا العينات التي عوملت بالسلق في الماء المغلي لمدة ٣,٥ دقيقة.

تحكيم: ا.د مجدي جمعه الشيمي
ا.د شاكر شحاتــه رزق