TOTAL ANTIOXIDANT ACTIVITY (TAA) OF BELL PEPPER DURING PROLONGED STORAGE ON LOW TEMPERATURE

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Abstract: Bell pepper (Capsicum annum L.) is a vegetable known for its antioxidant content, highly important for its nutritional values. The total antioxidant activity (TAA) of pepper fruits is measured by TEAC (Trolox equivalent antioxidant capacity). This assay measures both the hydrophilic (HAA) (vitamin C) and lipophilic (LAA)(carotenoids and vitamin E) contents based on the total radical scavenging capacity and the ability of a scavenger the stable ABTS radical (ABTS⁺) described by Vinocur and Rodov (2006). Fruit were cleaned and disinfected with hot water by rinsing and brushing (HWRB) at 55°C as it is described by Fallik et al., (1999). Tap water wash was served as control. Fruit were stored at 2°C or 7°C during 3 weeks plus 3 days at 20°C (shelf life simulation).

TAA in red bell pepper, immediately after harvest, was 4.29 (0.74 lipophilic and 3.55 hydrophilic) mol TE/g fr.wt. After 3 weeks storage at 2°C, TAA in pepper with cold wash treatment was 4.14 and 3.97 TEAC mol TE/g fr.wt. in HWRB treatment.

After 3 weeks +3 days shelf life on 20°C TAA slowly growing up and obtained content of 5.24 in cold wash treatment was 4.14 and 3.97 TEAC mol TE/g fr.wt. in HWRB treatment. This is mainly due to changes in the lipophilic activity-LAA (treatment with cold water-1.79 and 1.81 mol TE/g fr.wt. in HWRB, comparing with 0.74 mol TE/g fr.wt. on beginning of storage). Hydrophilic antioxidant activity-HAA remains practically unchanged.

In fruit, stored at 7°C, pepper ripeness has been associated with carotenoids accumulation especially after shelf life, TAA was 5.33 TEAC ( LAA 2.03) mol TE/g fr.wt

Key words: storage, pepper, antioxidant, quality, low temperature
Introduction

Fresh fruit and vegetables are important sources of antioxidants in human diet. Bell pepper is one of the commercially important vegetable crops and may be green, purple (unripe), red, yellow, orange, or brown when ripe. Peppers are rich in both hydrophilic antioxidants, such as vitamin C, and lipophilic ones, such as carotenoids and vitamin E (Vinocur and Rodov., 2006) and in potassium, and are low in calories, (Maalekuu et all., 2003). The major post harvest limiting factor in bell pepper is its relatively short shelf life of only one to two weeks.

Red sweet pepper (Capsicum annum L.) cultivars have been identified as potential vegetables with high antioxidant activity. Fresh sweet pepper has exceptionally high level of ascorbic acid (0,15 to 2,0 mg g⁻¹ fresh weight) compared to other fruit and vegetables. The attractive red color is due to the various carotenoid pigments. Ascorbic acid development in pepper and other fruit is related to glucose metabolism and light exposure, and concentrations of both ascorbic acid and reducing sugars typically increased as the fruit matures, (Fox et.all., 2005). Ascorbic acid was the main form of vitamin C, and its content increased as the pepper reached maturity. The ascorbic acid content increases in peppers as they ripen. For green mature, breaker and red peppers values of 107.3, 129.6 and 154.3 mg/100g edible portion was found, (Martinez et al., 2005). The red ripe stage had a relevant impact on the carotenoids content of polyphenols, while red ripe fruits had the highest content of vitamin C and provitamin A, (Marin et all., 2004).

Bell pepper are increasingly harvested at full color due growing consumer demand for peppers with improved flavor and nutritional aspects (Frank et al., 2001).

The vitamin C content for green mature and breaker peppers stored on room temperature (20°C) increased up to 10 days of storage, reaching similar values as those obtained for red peppers direct from the plant. However, stored red ripe peppers showed a significant loss in vitamin C content, around 25%. Refrigeration at 4°C for up to 20 days not change the ascorbic acid content, except for red peppers, which have showed losses around 15% (Martinez S., et all, 2005).

Antioxidants protect against free radicals and they are therefore important in obtaining and preserving good health. Much attention has been given to the flavonoids, a class of polyphenols with strong antioxidant activities (Arts et al., 2003).

Total antioxidant capacity (TAC) of 32 fruit could be divided into four groups: the first group, containing highest antioxidant capacity (>10mmol Trolox/g) included persimmon, strawberry, guava and pomegranate, the second group contained high antioxidant capacity between (5-10 mmol Trolox/g) included kiwi fruit, sweet cherry, fuji apple etc.), the third group containing medium antioxidant capacity (3-5 mmol Trolox/g) included orange, nectarine, banana, tomato; and fourth group containing lower antioxidant capacity(< 3mmoleTolox/g), include melon, pineapple honeydew melon, grape, pear and watermelon (Lang,Yow-shen, Ke,Lih-shang, 2006). Values of antioxidant activi-
ties measured by the TEAC methods are reported to have high antioxidant activities in brocoli-6.48, onion-5.32, cauliflower-2.95, tomato-2.55 and lettuce 1.71mol g\(^{-1}\) on a fresh weight basis (Bahorun et al., 2004).

The method of Vinokur and Rodov 2006, was applied for evaluation of total, hydrophilic and lipophilic antioxidant activity in various fruits and vegetables. In avocado, the ratio of lipophilic and hydrophilic antioxidants varied in different cultivars from 1:1 to 1:3. In cherry tomatoes this ratio changed from approximately 1:5 in green fruit to 1:3 in pink one and 1:1,5 in red one. Opposite to total activity increased from 0,6 to 1,5 M trolox equivalents (TE) per gram of fresh weight, most probably due to the accumulation of lycopene. Similar trends in changes of lipophilic and hydrophilic antioxidant activities in ripening tomatoes were described by Cano et al., 2003. The total antioxidant activity in strawberries has far exceeded that in other object tested, reaching the level of 14M TE/g. At the same time, practically all antioxidant activity of strawberry was represented by hydrophilic compounds with just trace amount of lipophilic antioxidants Vinocur and Rodov., 2006.

The aims of this research were to evaluate the keeping quality (content of total antioxidant activity, water loss, firmness, and decay incidence) of bell sweet pepper held for three weeks storage on low temperature using pre storage heat treatment and individually shrink-wrapped fruits.

**Material and Methods**

Red bell pepper (Capsicum annuum L.), cv. Selica was grown in the plastic house (Jordan Valley - Israel) under winter conditions. Fruit were harvested in March at the ripe stage (11,4 % dry matter) of uniform size and color. After harvest fruit were placed on commercial machines for cleaning and disinfecting pepper fruit. A unique and rapid method (Fallik et al., 1996) for simultaneously rinsing and disinfecting of (Israeli patent 116965) sweet pepper by using hot water (55+/- 1°C for 12+/-2s). Quality parameters were evaluated immediately after harvest and at the end of 21 days storage at7°C and 2°C plus three days at 20°C (shelf life simulation).

Control-unheated fruits have been washed only in cold water were also placed at 2°C. Half of this fruits were individually shrink-wrapped with Cryovac D-955 film and stored also at 2°C with relative humidity of 90% (R.V.) during three weeks.

Weight loss was expressed as percentage of weight loss from the initial weight of five fruits. Total soluble solid was measured using a digital refractometer. Fruit color was assessed by Minolta colorimeter, for fruit firmness use method described by (Ben-Yehoshua et al., 1983), CI- chilling injury measured visually as surface lesion on the fruits. Fruit was considered decayed once fungal mycelia appeared on the peel or calyx.
Total antioxidants activity (hydrolytic and lipophilic) was evaluated as scavenging capacity towards by measuring the decolorization of the cation radical of 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS\(^{+}\)) and expressed as Trolox equivalent antioxidant capacity (TEAC) (Vinokur and Rodov, 2006). The radical was generated in acidified ethanol medium in order to allow the performance of both hydrophilic and lipophilic antioxidants. The 2,2\(^1\)-azobis(2-amidinopropane) dihydrochloride (AAPH) was employed as a radical initiator. The reaction mixture for the generation conditions were optimized by choosing an appropriate acidifier, its concentration and incubation time. Incubation of the reaction mixture at 45°C for 75 minutes was sufficient for ABTS\(^{+}\) generation. The obtained stock solution of ABTS\(^{+}\) could be stored at least 2 days at 4°C without significant loss of its properties.

The extraction procedure was developed to allow isolation of hydrophilic and lipophilic fractions from fresh fruits or vegetables without preliminary drying. The procedure was based on stepwise extraction of the plant material with acetate buffer, acetone and hexane and repeated partition of water-soluble and water-insoluble portions. At first stage, the fresh plant material was homogenized in acetate buffer pH 4.3 and the homogenate extracted/dehydrated with three successive portions of acetone, each step followed by centrifugation and collection of the supernatant. The acetone-water supernatant fractions from the three extractions were pooled. After the acetone extraction, the dehydrated pellet was extracted three times with hexane and supernatants collected and pooled. The traces of lipophilic compounds were extracted from the pooled acetone-water extract by partitioning with hexane. The non-polar fraction obtained from this operation was subjected to another partition with water in order to extract the traces of hydrophilic compounds. After pooling the fractions, two samples were obtained, hydrophilic (water/acetone extract), and lipophilic (hexane extract). Until the partition stage, all the extraction steps for one plant sample were performed in a single centrifuge test tube; the partition required two test tubes per plant sample.

The discoloration test was performed in plastic cuvettes by adding 10L of test sample to 1mL of acidified ethanolic solution of ABTS\(^{+}\) and measured as optical density at 734nm after 15 min of incubation at room temperature in comparison with blank sample. The 1mM solution of 6-hydroxy -2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox a water-soluble derivate of the vitamin E) was used as a standard, and the radical-scavenging activity of samples was expressed as Trolox equivalent antioxidant capacity (TEAC).

The antioxidant activity of the measured extract in Trolox equivalents (TE, \(\mu\)M) is calculated as follows:

\[
TE = C_{\text{standard}} \cdot \frac{(A_{\text{sample}} - A_{\text{blank}})}{(A_{\text{sample}} - A_{\text{blank}})}
\]

where \(C_{\text{standard}}\) is standard concentration (usually equal 1mM), \(A_{\text{sample}}\), \(A_{\text{blank}}\) and \(A_{\text{standard}}\) are absorbance values of the samples, blank and standard, accordingly.
For example, if obtained TE value is equal 0.5 mM it means that 1 ml of the measured extract has the same antioxidant activity as 1 ml of Trolox solution of the concentration 0.5 mM.

For calculating Trolox equivalent antioxidant capacity (TEAC) in plant tissue per weight unit, the extract volume V and the weight of the extracted tissue M should be taken into consideration in the:

$$\text{TEAC (mM TE/g) = (TE \cdot V) / (1000 \cdot M)}$$

All the results were subjected to statistical analysis using Duncan's multiple test analyses of variance (ANOVA) computer program.

**Results and Discussion**

Antioxidant activity of bell peppers depends on several factors including genetic, environmental condition (temperature, light, water, and nutrient availability), production techniques used (plant grow regulators, date of harvest, etc.) and post harvest storage conditions.

It is necessary to know more about the effects of post harvest conditions, especially of low temperature on antioxidants in pepper, because temperature is the main factor for pepper quality in terms of antioxidants.

The relative capacity of antioxidants for scavenging ABTS$^+$ radical was compared to the antioxidant potency of Trolox (water-soluble vitamin E) as standard. TAA in red bell pepper immediately after harvest was 4.89 (0.84 lipophilic and 4.05 hydrophilic) mol TE/g fr.wt. After 3 weeks storage at 2°C, TAA in pepper with cold wash treatment was 4.72 and 3.97 TEAC μmol TE/g fr.wt. in HWRB treatment.

**Tabela 1. Variation of antioxidant activity during 3 weeks storage on low (2 or 7°C) followed by 3 day on 20°C (shelf life) as expressed by TEAC values (μmol TE/g fresh weigh).**

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Lipophilic</th>
<th>Hydrophilic</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lipophilic</strong></td>
<td>TE</td>
<td>TEAC</td>
<td>TEAC</td>
</tr>
<tr>
<td><strong>Hydrophilic</strong></td>
<td>TEAC</td>
<td>TEAC</td>
<td>TEAC</td>
</tr>
<tr>
<td><strong>Start T0</strong></td>
<td>TEAC</td>
<td>TEAC</td>
<td>TEAC</td>
</tr>
<tr>
<td><strong>After 3 week storage on 7°C</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7°C T&lt;sub&gt;21&lt;/sub&gt;</td>
<td>0.10</td>
<td>1.55</td>
<td>0.29</td>
</tr>
<tr>
<td>7°C T&lt;sub&gt;21+3&lt;/sub&gt;</td>
<td>0.15</td>
<td>2.51</td>
<td>0.31</td>
</tr>
<tr>
<td><strong>After 3 weeks storage on 2°C</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2°C Cold Water - T&lt;sub&gt;21&lt;/sub&gt;</td>
<td>0.09</td>
<td>0.70</td>
<td>0.29</td>
</tr>
<tr>
<td>2°C C.W - shrink - T&lt;sub&gt;21&lt;/sub&gt;</td>
<td>0.09</td>
<td>1.40</td>
<td>0.26</td>
</tr>
<tr>
<td>2°C HWRB - T&lt;sub&gt;21&lt;/sub&gt;</td>
<td>0.08</td>
<td>1.12</td>
<td>0.30</td>
</tr>
<tr>
<td>2°C HWRB+shrink - T&lt;sub&gt;21&lt;/sub&gt;</td>
<td>0.08</td>
<td>1.08</td>
<td>0.29</td>
</tr>
<tr>
<td><strong>After 3 weeks on 2°C + 3 day on 20°C (shelf life)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2°C Cold Water - T&lt;sub&gt;21+3&lt;/sub&gt;</td>
<td>3.04</td>
<td>0.25</td>
<td>3.93</td>
</tr>
<tr>
<td>2°C C.W - shrink - T&lt;sub&gt;21+3&lt;/sub&gt;</td>
<td>0.10</td>
<td>1.80</td>
<td>0.26</td>
</tr>
<tr>
<td>2°C Hot.Water,K.B - T&lt;sub&gt;21+3&lt;/sub&gt;</td>
<td>0.14</td>
<td>2.06</td>
<td>0.32</td>
</tr>
<tr>
<td>2°C HWRB+shrink - T&lt;sub&gt;21+3&lt;/sub&gt;</td>
<td>0.11</td>
<td>1.87</td>
<td>0.30</td>
</tr>
</tbody>
</table>
After 3 weeks +3 days shelf life on 20°C TAA slowly growing up and obtained content of 5.97 in cold wash treatment and 5.88 TEAC μmol TE/g fr.wt. in HWRB. This is mainly due to changes in the lipophilic activity-LAA (treatment with cold water-2.04 and 2.06 μmol TE/g fr.wt. in HWRB, comparing with 0.84 μmol TE/g fr.wt. on beginning of storage). Hydrophilic antioxidant activity-HAA remains practically unchanged.

In fruit was stored at 7°C pepper ripeness has been associated with carotenoids accumulation especially after 3 days on room temperature (shelf life), TAA was 5.33 TEAC (LAA 2.03) μmol TE/g fr.w.

Increasing TAA during low temperature storage could be related to ripening processes and metabolism of phenolic compounds Javanmardi and Kubota., 2006. During ripening, the TAA increases and this increase is mainly due to changes in the LAA. HAA remains practically unchanged after 3 week on 2°C (between 2-5%) from the initial value. Similarly, Martinez et al., (2005) published that during storage of red pepper at 4°C for up to 20 days showed losses in ascorbic acid content around 15%.

The new method was applied for evaluation of TAA (HAA and LAA) in various treatment on low temperatures on pepper fruits during prolonged storage. In pepper fruit this ratio between HAA and LAA was 1:2 and 1: 3, depend of storage temperature and postharvest treatment (heat treated and wrapped fruit). In investigations Vinocur and Rodov., (2006) in cherry tomatoes this ratio changed from approximately 1:5 in green fruit to 1:3 in pink one and 1: 1,5 in red one. In parallel, the total activity increased from 0.6 to 1.5 μM Trolox equivalents (TE) per gram fresh weight, most probably due to the accumulation of lycopene. Similar trends in changes of lipophylic and hydrophillic antioxidant activity in ripening tomatoes were described by Cano et al., (2003). Practically all antioxidant activity of strawberry was represented by hydrophillic compounds with just trace amount of lipophilic antioxidants.

Temperature stresses before storage (hot water 55°C for 12 sec) and low temperature during storage conditions (3 weeks on 2°C) affect the pathways involved in the biosynthesis secondary metabolites, lead to higher phenolic metabolism and antioxidant capacity on pepper fruit.

Tab. 2 : Total carotenoid content (mg/g) in pepper fruit during storage period

<table>
<thead>
<tr>
<th>Content of carotenoides</th>
<th>mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start T&lt;sub&gt;0&lt;/sub&gt;</td>
<td>0.10</td>
</tr>
<tr>
<td>7°C T&lt;sub&gt;21&lt;/sub&gt;</td>
<td>0.17</td>
</tr>
<tr>
<td>7°C T&lt;sub&gt;21+3&lt;/sub&gt;</td>
<td>0.29</td>
</tr>
<tr>
<td>2°C Cold Water -T&lt;sub&gt;21&lt;/sub&gt;</td>
<td>0.13</td>
</tr>
<tr>
<td>2°C C.W +shrink -T&lt;sub&gt;21&lt;/sub&gt;</td>
<td>0.12</td>
</tr>
<tr>
<td>2°C HWRB - T&lt;sub&gt;21&lt;/sub&gt;</td>
<td>0.11</td>
</tr>
<tr>
<td>2°C HWRB+shrink-T&lt;sub&gt;21&lt;/sub&gt;</td>
<td>0.10</td>
</tr>
<tr>
<td>2°C Cold Water -T&lt;sub&gt;21+3d, shelf life&lt;/sub&gt;</td>
<td>0.22</td>
</tr>
</tbody>
</table>
A total carotenoid content of bell peppers after 3 week of storage on 2°C remains practically unchanged (especially fruit from hot water treatment and wrapped fruit - 10mg/g) in comparing with 0,10mg/kg on beginning of storage. Content in cold water fruit was 0,13mg/g. In same period carotenoid content in fruit storage at 7°C was 0.17mg/kg fresh weight.

After shelf life period (adding 3 day on 20°C) the carotenoid content was significant increased as much as at 7°C (0.29mg/g f.w.) in compare to fruit on 2°C (0,21-0,22 mg/kg f.w.). In fruit was stored at 7°C pepper ripeness has been associated with carotenoids accumulation especially after 3 days on room temperature – during shelf life period.

Tab.3: Quality parameters of red bell pepper after prolonged storage on low temperature

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Water loss%</th>
<th>Firmness (1-4)</th>
<th>Decay % fruit</th>
<th>Decay % calyx</th>
<th>Color (1-4)</th>
<th>Chilling injury - CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>HWRB – 7°C</td>
<td>3.31</td>
<td>3.2</td>
<td>18.2</td>
<td>6.0</td>
<td>3.5</td>
<td>0</td>
</tr>
<tr>
<td>Cold water – 2°C</td>
<td>3.95</td>
<td>2.6</td>
<td>30.5</td>
<td>23.6</td>
<td>3.25</td>
<td>78.5</td>
</tr>
<tr>
<td>HWRB</td>
<td>4.24</td>
<td>2.5</td>
<td>28.3</td>
<td>25.2</td>
<td>3.5</td>
<td>53.5</td>
</tr>
<tr>
<td>Cold water +shrink</td>
<td>0.47</td>
<td>2.2</td>
<td>14.5</td>
<td>7.2</td>
<td>3.25</td>
<td>9.2</td>
</tr>
<tr>
<td>HWRB+shrink</td>
<td>0.56</td>
<td>2.0</td>
<td>13.2</td>
<td>7.9</td>
<td>3.0</td>
<td>8.1</td>
</tr>
</tbody>
</table>

Shelf life begins after harvest, therefore maintaining a low rate of weight loss and softening in bell pepper after harvest is important for prolonged storage and sea transport to distant markets. Fruit quality can be characterized by several interrelated factors, such as general appearance, firmness and decay incidence. The general appearance (GA) of fresh bell peppers, firmness and texture are important criteria for determining market quality and consumer acceptance. Quality of bell pepper after harvest is largely influenced by water loss from the fruit. About 26% of the water loss in mature fruit occurred through the calyx (Diaz-Perez et al., 2006). Fruit water loss was positively correlated with degree of chilling injury.

Damage caused by chilling injury in peppers is typified by dot-pitting followed by sheet-pitting. The degree of chilling injury depends on the temperature to which it is exposed, the duration of exposure, maturity and the part of the fruit. Hot water treatment and shrink film wrapping decreases percent of chilling on bell pepper fruit during storage on low temperature. The individually shrink-
wrapped pepper fruit could be stored for 3 weeks on 2°C followed pre storage treatments with cold and hot water rinsing.

In same time non wrapped fruit from same treatment, showed big percentage of CI (chilling injury) symptoms (78.5% and 53.5%) during shelf life period (3 days on 20°C). The wrapped fruits ripened normally with good sensory traits (with firm texture and good flavor), during shelf life period when shifted to 20°C after unwrapping.

Steam and calyx are more sensitive fruit part in comparison with fruit mesocarp. These morphological lesions may lead to Alternaria-induced rot on pods and calyxes, seed darkening, and fruit shrinkage due to moisture loss. This chilling induced fruit damage and reduces the quality and shelf life of pepper fruit.

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Literatura:


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UKUPNA ANTIOKSIDATIVNA AKTIVNOST (TAA) PAPRIKE TOKOM DUŽEG ČUVANJA NA NISKIM TEMPERATURAMA

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Rezime


TAA kod crvene paprike tipa babure neposredno nakon berbe je 4.29 (0.74 lipofilni i 3.55 hidrofilna aktivnost) mol TE/g sveže mase. Posle tronedeljnog čuvanja na 2°C, TAA kod paprika tretmanom pranja hladnom vodom je 4,14 i 3.97 TEAC mol TE/g kod HWRB.

Posle 3nedelje+3dana na 20°C TAA blago raste i ostvaruje sadržaj od 5.24 u tretmanu hladnom vodom i 5.16 TEAC mol TE/g sv. mase kod HWRB. Ovo po-većanje se uglavnom dešava tokom promena u lipofilnoj aktivnosti (tretman sa hladnom vodom 1.79 i 1.84 µmol TE/g sveže mase kod HWRB, u poredenju sa 0.74 mol TE/g sv. mase početkom čuvanja. Hidrofilna antioksidativna aktivnost praktično ostaje nepromenjena.

Kod plodova čuvanih na 7°C zrenje je udrugeno sa akumulacijom karotenoida posebno nakon shelf-life perioda, TAA je 5.33 (lipofilna faza 2.03) µmol TE/g sveže mase.

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