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Effect of Cold Storage and Modified Atmosphere Packaging on Strawberry (Fragaria X Ananassa Duch.) cv. "Arben" Fruit Keeping Quality

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Strawberry is one of the most popular fruits worldwide. Therefore, it is important to maintain its supply and freshness and potency guaranteed from the farms to the point of consumption. Strawberries spoil rapidly and the quality changes with storage delays. However, little is known about the effect of storage methods on the changes of its quality. In this study, two storage methods; Cold Storage (CS) and Modified Atmosphere Packaging (MAP) were investigated to see how it will maintain its quality. Results obtained showed that storage method has an effect on keeping its quality, and it proved that MAP is the acceptable storage method because it kept on strawberry quality, extended storage period, and decreased weight loss, in contrast, showed the highest fruit decay, in compare to the cold stored fruits. And that means; MAP can be useful supplements to provide optimum storage conditions (temperature and relative humidity) and maintain the quality of fresh strawberry fruits after harvest.

Keywords: Vitamin C; Total Phenolic; Anthocyanin; Titratable Acidity; Soluble Solids.

Strawberry (*Fragaria* X *Ananassa* Duch.), which is flavorful and fragrant, is one of the most popular fruits worldwide (Abu-Zahra and Tahboub, 2008a). In addition, the fruit having high antioxidant activities thus contains large quantities of ascorbic acid (vitamin C), anthocyanins and other organic compounds (Cordenunsi *et al*., 2005; Kalt *et al.,* 1999; Klopotek *et al*., 2005). Although, anthocyanins are responsible for the fruit red color (Nielsen *et al*., 2003), regarded as a natural alternative to replace synthetic food colorants and as a plant phenolic compounds (Serrano *et al*., 2009).

Strawberry pre-cooling is very crucial, because it is very brittle and its quality degradation occurs very quickly prior to shipping, researchers found that strawberry fruit deteriorate faster than any other fruits (Anderson *et al*., 2004; Kitazawa *et al.,* 2013). Precooling could be applied by hydro-cooling, forced-air cooling, or room cooling (Ferreira *et al*., 1994). Although, strawberry fruits could be stored under controlled atmosphere (CA), modified atmosphere packaging (MAP) or under cold air storage (Abu- (Abu-Zahra and Tahboub, 2008b). But according to Smith (1992), the longest storage period could be obtained by CA; in which O_2 concentration is reduced, CO_2 concentration is increased, in addition to low temperature storage conditions, and these storage conditions could enhance fruit quality and decrease decay, which

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attributed to CO_2 suppressing fungal growth (Smith, 1992).

Packaging material affects the quality and shelf life. Packing in polyethylene bags decreased respiration, maintained quality, prolonged the shelf life (Krivorot and Dris, 2002). In addition, packaging protects against mechanical damage, and prevents moisture loss (Perez *et al*., 1997). However, non-perforated films significantly increased shelf life, improved fruit firmness, and reduced weight loss and rot. Detrimental fruit quality loss can be reduced by appropriate handling and use of suitable fruit packaging during storage, because it inherits modified atmosphere (MA) (Manleitner *et al*., 2002). Weight loss of non-packaged berries was 2.0-4.0 % per day, whereas losses in packaged fruits were only 0.05-0.63 % per day (Krivorot and Dris, 2002).

Respiration rate increased under air storage conditions at 5 °C; at the beginning of storage respiration rate was 6-9 mg CO_2/kg.hr, and reached 7-14 mg CO_2/kg.hr at the end of storage, as found by Pelayo *et al*. (2003). Morris *et al*. (1985) reported that as post harvest holding time and temperature increased the quality of strawberries decreased, and storage under low temperature was reported to be the only way to minimize ascorbic acid degradation rate (Castro *et al*., 2004).

According to Shiina (2003), Berry fruits commercial value will be lost, if 5 % or more reduce its water content. However, in Kitazawa *et al.* (2013) study, only 1.7 % of Berry fruit water content -after storage- was lost. Wrapping Berry fruits with polyvinyl chloride (PVC) film kept on fruit firmness and total titratable acidity, while reduced the total soluble solids content (Ferreira *et al*., 1994). While, in another storage experiment conducted by Kitazawa *et al.* (2013), total soluble solids and ascorbic acid (vitamin C) of berry fruits were decreased after storage.

Storage temperature influence to a large extent the physiological and biological changes taking place in the strawberry fruits (Pathare *et al*., 2012), and according to Ravindra and Goswami (2008), temperature is considered as the most important factors affecting the vase life of strawberry fruits. On the other hand, bad fruit handling after harvest could reduce fruit keeping quality and decrease its postharvest life (Abu-Zahra and Tahboub, 2008b).

Strawberries are perishable fruit and very sensitive to microbial decay; gray mold or botrytis fruit rot (caused by *Botrytis cinerea*) are the most important fruit rots of strawberry. Bad management of storage temperatures, can easily damage strawberry fruit and increase their vulnerability to infection by decay pathogen (Nunes *et al*., 2005).

In Jordan; most of strawberry fruits are produced under greenhouse conditions as soilless culture (mixture of peatmoss and perlite), with little direct planting in raised beds. According to Ministry of Agriculture (2015), more than 350 ha of strawberry plants are planted in Jordan every year. Strawberry fruits are an extremely valuable crop, but they are susceptible to mechanical damage and water loss, if not cooled and maintained at proper temperatures. Little is known about the effect of storage methods on the changes in fruit keeping quality. In this study, two storage methods; Cold Storage (CS) and Modified Atmosphere Packaging (MAP) were investigated on the strawberry to study the changes that may occur in the fruit content after storage.

MATERIALS AND METHODS

An experiment, were carried out with strawberry fruits (*Fragaria* X *Ananassa* Duch.) variety "Arben", grown in the Madaba city at Al-Tamimi Farms, and produced under soilless conditions (peatmoss and perlite mixture). Strawberry fruits were harvested from September to December/2016; fruits were selected for uniformity of size and color, freedom from soil, mechanical damages and defects. The harvested fruits were stored at 3 °C and about 90-95 % R.H., under two storage conditions; the first storage method is the Cold Storage (CS); in which fruits were packaged in un-covered small plastic containers (500 g capacity), stored under low temperature (3 °C). The second storage method is the Modified Atmosphere Packaging (MAP); in which fruits were packaged in the same containers capacity, but in this treatment, containers were wrapped with a shrink-wrap (manufactured in Al-Nur Jordanian company for plastic industry, Amman-Jordan) is made from LLD-polyethylene resin, approved as pollution-free.

Containers were filled with about 450 g fresh strawberry fruits and stored uncovered (cold

storage treatment) or covered with shrink-wrap (MAP treatment). The storage experiments were repeated at four harvesting dates (four replications), at each storage time: four containers were used for each treatment, and at each time average readings were considered per treatment.

Before storage; gases of the storage room were measured which was: 20 % Oxygen (O_2) and 0.033 % Carbon dioxide (CO_2) using Respirometer instrument (produced by Engineering 360 IEEE Global space company), and at the end of the experiment, gases (O_2 and CO_2) were determined for the storage room and for the wrapped fruits.

To avoid an accumulation of ethylene inside the wrapped containers, an ethylene adsorbing monolayer was added, according to Steen *et al*. (2002) recommendations. On the other hand, humidity in the cold room was controlled by Humidifier (Ultrasonic Humidifier Cole-Parmer Inst. Co., Order No. U-37700-85, USA), and by water addition (two times per day) to the storage room floor (which kept to be around 95 %).

Parameters Measured

Each fruit container was removed from the storage refrigerator, when fruits started showing wilting symptoms or any rot incidence. Measurement or analyses of samples for all parameters were done four times during the four months of fruit harvesting.

Estimation of Storage Period

Number of storage days was estimated at the end of the storage periods, when berry fruits start showing wilting symptoms, but still acceptable for eatings, by counting the number of days from the first day of storage until the end of the storage period.

Estimation of Weight Loss

Before storage; strawberry fruit weight in each container was measured, then at the end of the storage period the weight loss was calculated and recorded for each replicate.

Estimation of Rotted fruits

At each container, when berry fruits starts showing wilting or rot symptoms, storage was ended and number of rooted fruits were counted and recorded for each replicate.

Estimation of Soluble Solids and Titratable Acidity %

They were estimated, before storage and at the end of each replicate storage method. The analysis or measurements were applied according to Sahari *et al*. (2004); and Abu-Zahra and Tahboub (2009) procedures; strawberry fruit samples were grounded and filtered through a cloth sheet; then few drops were used to determine the soluble solids by using the refractometer instrument. On the other hand, a five ml of the filtrate were used to determine the titratable acidity by doing a titration with 0.1 N NaOH.

Fruit Anthocyanins

It was estimated before storage and at the end of the storage period by pH-Differential Spectrophotometer method, absorbance was taken by Spectrophotometer; The fruits were grinded and homogenized through pestle and mortar. Then a 10 g sample was placed in a plastic bottle, 100 ml of 1: 99 V/V (HCl: methanol) added to it and shacked for half an hour, then filtrated through filter paper, two samples five ml each were placed in two volumetric flasks (25 ml) and the volume for each flask, was completed with a different pH buffer (pH 1 for sample 1 and pH 4.5 for sample 2). The absorbance was measured for each of the samples at the wavelengths 510 and 700 nm. After that, the following equation was applied:

Absorbance = $(A_{510\ nm}\ pH\ 1 - A_{700\ nm}\ pH\ 1) - (A_{510\ nm}\ pH\ 4.5 - A_{700\ nm}\ pH\ 4.5)$

Then total anthocyanins (% W/W) in the samples were calculated according to the following equation: $\%\ W/W = A/L \times MW \times DF \times V/Wt \times 100$.

Where:

A = Absorbance.

= Pelargonidin 3-Glucoside (PGD 3-GLU) coefficient = 22400.

L = Cell path length (usually 1 cm).

MW = Anthocyanin molecular weight = 433.2

DF = Dilution factor.

V = Final volume (ml).

Wt = Sample weight (mg).

Then the obtained values (g/100 g) were converted into mg anthocyanin/100 g fruit fresh weight by multiplying with 1000. Finally, the results were expressed as milligrams of pelagonidin-3-glucoside per 100 g fruit fresh weight. According to the procedures used by Abu-Zahra *et al*. (2007a&b).

Ascorbic Acid (Vitamin C)

It was measured before and at the end of the storage methods by grinding 5 g of composite fruit tissue per replicate with 40 ml of

5 % metaphosphoric acid, and then homogenized for 4 minutes with pestle and mortar; and filtered through filter paper. Next, the filtrate was diluted to 100 times with distilled water, and ascorbic acid was measured by classical titration method using 0.2 % of 2, 6-Dichlorophynol indophenol's solution (DCPIP). Finally, the ascorbic acid concentration was expressed as mg ascorbic acid/100 g fresh weight according to the following equation:

$$\text{Ascorbic acid} \quad \text{ml DCPIP used} \times D_f \times \text{Dilution factor/Sample weight (g)}$$

D_f (Ascorbic acid and DCPIP equivalent amount) = 1 mg ascorbic acid/22.5 ml DCPIP
(Sahari *et al*., 2004; and Klopotek *et al*., 2005).

Total Phenolic

This parameter was determined according to methods used by Asami *et al*. (2003); and Pelayo *et al*. (2003). Fresh strawberry fruits were grinded and homogenized with pestle and mortar, 3 g aliquot was transferred to beakers and extracted with 40 ml of a mixture containing acetone, water, and acetic acid (70:29.5:0.5). Samples were vortexed with Vortex Mixer (Biosan, V1 Plus-Spain) and allowed to stand for 1 h at room temperature for complete solvent extraction. Extracts were centrifuged at 4000 round/minute for 15 minutes using Hettich EBA-20, Tuttlingen-Germany a centrifuge, then filtrated and concentrated under partial vacuum at 40 ºC using a rotary evaporator (Heidolph, Laborota 4001, Schwabach-Germany). Then the samples were brought up to a total volume of 25 ml. TP concentrations were measured using the Folin-Ciocalteu assay (Sigma, Steinheim-Germany), 0.5 ml of sample, and 0.5 ml of Folin-Ciocalteu reagent were added to a 25 ml volumetric flask, mixed and allowed to stand for 5-8 min. Then 10 ml of a 7 % sodium carbonate solution was added, followed by the addition of distilled water to reach the desired volume. Solutions were mixed and allowed to stand for 2 h, and then TP concentration was measured using a spectrophotometer (Biotech Engineering Management CO. LTD., UV-9200, Nicosia-Cyprus) monitoring 750 nm. The results were expressed as milligrams Gallic acid equivalent (GAE) per 100 g fresh weight according to the following equation:

$$\text{TP} \quad \text{Concentration} \times 25 \text{ ml} \times \text{Dilution factor} \times 100 \text{ g}/1000 \times \text{Sample weight (g)}$$

Dilution factor = 50/1

Statistical Analysis

The experiment design was A Completely Randomized Design (CRD), were used with two treatments: Cold storage (CS) and Modified Atmosphere Packaging (MAP). Each treatment repeated at four storage times (four replications); at each storage time, four plastic containers were used per treatment. The data were subjected to analysis of variance (ANOVA), according to procedures outlined by Steel and Torrie (1980). Mean separation was conducted by the Least Significant Difference (LSD) using SAS program. Differences with probability value equals to 0.05 were considered significant.

Table 1. Results of strawberry fruit quality measurements before storage

No.	Parameters measured	Readings
1.	Ascorbic Acid	54.07 (mg 100g f wt^{-1})
2.	Anthocyanin	39 (mg 100g f wt^{-1})
3.	TSS %	7.42 %
4.	TTA %	0.83 %
5.	Total phenols	1129 (mg 100g f wt^{-1})

Table 2. Results of strawberry fruit quality measurements after storage:

Storage type	Ascorbic Acid (mg 100g f wt^{-1})	Ascorbic Acid change (%)	Anthocyanin (mg 100g f wt^{-1})	Anthocyanin change (%)
*CS	49.3 a***	-8.8	47.2 b	+ 21
**MAP	53.5 a	-1.05	56.2 a	+ 44
$LSD_{0.05}$	4.8		7.2701	

*CS: Cold Storage; **MA: Modified Atmosphere Packaging; *** Means within each column having different letter(s) are significantly different according to LSD at 5% level.

RESULTS AND DISCUSSION

Composition of Gases

At the end of the storage period; gases (O_2 and CO_2) were measured; results obtained do not show any change in the storage room gases, which remained constant (20 % O_2 and 0.033 % CO_2), because the storage room doors were open every day to check the samples, while MAP storage method reduced levels of oxygen (5.5 %), elevated concentrations of carbon dioxide (12 %), and maintained humidity around the fruits. The decrease in oxygen and increase in carbon dioxide in MAP benefits include reduced respiration, retarded softening and compositional changes; and reduced decay.

According to Kader *et al*. (1989), subjecting fresh produce to too low an oxygen concentration and/or to too high a carbon dioxide level can result in MA stress. Atmosphere modification within such packages depends on film permeability, commodity respiration rate and gas diffusion characteristics, and initial free volume and atmospheric composition within the package.

Fruit Quality measurements

Before storage; strawberry fruit samples were measured or analyzed for fruit quality determination, to compare with that at the end of the storage periods, results obtained were summarized at Table 1.

Vitamin C

The water-soluble vitamin C, did not show any significant differences between the used storage methods (Table 2.), but results showed a decrease in the vitamin C content during storage, comparing with measurements before storage as summarized in Table 1. The biggest losses in vitamin C (8.8 %) were observed in the cold storage treatment, while MAP caused only 1 % losses.

Investigations of vitamin C (Table 2.), proved that wrapping strawberry fruits protect their content from vitamin C more than unwrapped fruits, even though the differences are not significant. Kitazawa *et al*. (2013) obtained the same results, in which ascorbic acid of strawberry fruits were decreased after cold storage.

Total Anthocyanin

Before storage; the total anthocyanin content was 39 mg 100 g f wt^{-1} (Table 1.), then, after storage it increased to be 47.2 and 56.2 mg 100 g f wt^{-1} in cold storage and MAP, respectively, (Table 2.) with a statistical differences between the used storage methods.

Table 3. Results of strawberry fruit quality measurements after storage

Storage type	TSS (%)	TSS change (%)	TTA (%)	TTA change (%)	Total phenols (mg 100g f wt^{-1})	Total phenols change (%)
*CS	6.54 b***	-11.9	0.61 b	-27	881 a	-22
**MAP	7.32 a	-1.3	0.72 a	-13	882 a	-21.9
$LSD_{0.05}$	0.5751		0.0469		91.617	

*CS: Cold Storage; **MA: Modified Atmosphere Packaging; *** Means within each column having different letter(s) are significantly different according to LSD at 5% level

Table 4. Results of strawberry fruit quality measurements after storage

Storage type	No. of rotted fruits	Length of storage (Days)	Weight loss (%)
*CS	0 b***	15.25 b	0.36 a
**MAP	6.75 a	27.5 a	0.25 b
$LSD_{0.05}$	1.5395	5.2741	0.0776

*CS: Cold Storage; **MA: Modified Atmosphere Packaging; *** Means within each column having different letter(s) are significantly different according to LSD at 5% level.

Unwrapped fruits (cold storage) showed the lowest increase in total anthocyanin content with 21 % increase; compare to 44 % increase in total anthocyanin in wrapped fruits (MAP treatment) (Table 2.). These results proved that; anthocyanin pigment continue to synthesis during storage, even at low storage temperature conditions, and the highest increase in this pigment was obtained by the wrapped berry fruits, so these results are in agreement with those obtained by Pelayo *et al*. (2003), who found the same observations.

Total Soluble Solids (TSS) %

At the end of the storage periods, results showed significant differences in the measured soluble solids; the highest TSS % (7.32) were obtained by MAP treatment, while the lowest significant TSS % (6.54) were obtained by cold storage treatment (Table 3.). Comparing to measurements before storage (Table 1.); both storage methods showed a decrease in the TSS %, but the highest decease (11.9 %) was observed in uncovered fruits (Table 3.), while covered fruits kept on fruit TSS and showed only 1.3 % decrease after storage.

These results proved that wrapping fruits could decrease losses in fruit soluble solids during storage and that in agreement with Kitazawa *et al.* (2013), in which soluble solids of strawberry fruits were decreased after cold storage. On the other hand, in another study conducted by Ferreira *et al*. (1994), opposite results were observed; wrapped berry fruits with PVC film had lower soluble solids content after storage

Total Titratable Acidity (TTA) %

The total titratable acidity % was decreased from 0.83 before storage (Table 1.) to 0.61 and 0.72 in cold storage and MAP, respectively, (Table 3.). Cold storage showed the highest decline in the acidity compared to MAP that showed the lowest acidity decline. However, in other studies, outlined by Ferreira *et al*. (1994), total titratable acidy of wrapped berry fruits with PVC film was not affected after storage, which nearly coincides with results obtained in this research.

Total Phenolic

Higher amounts of total phenolic compounds were observed in the strawberry fruits with 1129 mg 100g f wt^{-1}, before storage, as summarized in Table 1. During storage process, this content was decreased in both storage methods (Table 3.); the decrease was about 22 % in both storage methods, without statistical differences between the used treatments.

The decrease in phenolic compounds under both storage methods proved that, the used storage methods (cold and modified storage) did not protected the phenolic compounds from decrease. And this coincides with findings obtained by Klopotek *et al*. (2005).

No. of Rotted Fruits

Only fruits stored under MAP storage conditions showed fruit decay (Table 4.), which is due to the long storage period with high relative humidity, because wrapping fruits kept on high humidity conditions around the fruits and encouraged fungi growth. While, uncovered fruits do not show any rotting incidence due to lower storage period and lower relative humidity. These results coincides with that obtained by Abu-Zahra (2007a), in which MAP produced the highest fruit decay compare to air stored fruits.

Length of Storage Period

The storage period was extended by using MAP storage method; since the storage period was extended from 15.25 days in uncovered cold stored fruits to 27.5 days in covered fruits (Table 4.).

Covering fruits with the shrink-wrap, maintained their quality by reducing moisture losses, decrease respiration, and so extended their vase life (Table 4.). These results are in agreement with that obtained by Krivorot and Daris (2002), who found that MAP of strawberry fruit extended the storage period more than air storage.

Weight Loss

Both storage methods showed a decrease in fruit weight at the end of the storage periods; a significant average weight loss, was observed in cold stored fruits with 0.36 % per day (Table 4.), while wrapping the fruits decreased their weight loss and kept on fruit quality.

Covering fruits with the shrink-wrap decreased the transpiration and respiration rate, compare to uncovered fruits that lost more weight due to the high transpiration and respiration rate (Table 4.). According to Shiina (2003), the commercial value of strawberry is lost when 5 % or more reduce its water content. In addition, same observations were obtained by Krivorot and Daris (2002), who found that: uncovered fruits loss more weight than covered ones.

CONCLUSIONS

In this study, we clarified the effect of storage methods on number of rotted fruits, length of storage period, weight loss, total anthocyanin, TSS, TTA and total phenolic in strawberry fruits. Packaging fresh produce in polymeric films can result in a commodity generated MA; results suggested that MAP is the acceptable storage method compare to cold storage, because it kept on strawberry fruit quality, extended storage period, and decreased weight loss, in contrast, it showed the highest fruit decay.

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