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# EFFECT OF ETHANOL AND SULFUR DIOXIDE FUMIGATION ON ANTHOCYANIN AND TOTAL PHENOLIC COMPOUNDS IN "HELWANY" TABLE GRAPE CULTIVAR DURING COLD STORAGE

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## ABSTRACT

Grape *Vitic vinifera* L.cv. "Helwany" was fumigated with absolute ethanol at two concentrations 75 and 100 % (8 ml ethanol $kg^{-1}$), also slow release sulfur dioxide generator pads (as sodium meta bisulfite,1 and 1.5 g. $kg^{-1}$) were applied on grape in 40 μm thicknesses polyethylene- lined boxes, fruit were stored at $0 \pm 1$ C° and 90-95 % RH in cool units of pome and vine research department in Sweida, and the experiment was performed in two successive season's 2008- 2009 intervals. The application of ethanol enhanced anthocyanin content (mg $100g^{-1}$) in berries skin. Also, total phenolic compounds (mg. $100g^{-1}$) in berries and in cluster rachis were better than control, but total phenolic compounds and berries respiration rate (mg $CO_2$. $kg^{-1}$. $h^{-1}$) decreased comparing to sulfur pads in two successive seasons.

**Key words:** Grape *Vitic vinifera* L., anthocyanin pigment, phenolic compounds, respiration rate, cold storage.

## INTRODUCTION

*Vitic vinifera* L.cv. "Helwany" is a late ripening, red seeded cultivar which highly profitable as it fills a niche gape in the market. It is one of the most table grape cultivars currently produced in Syria, and is widely cultivated in table grape producing regions, as in Arab mountain.

$SO_2$ used for the prevent of pathogens development especially gray mold after harvest and during the storage in the commercial application (Smilanick *et al.,* 1990), but this application effects on the berries quality, caused an residual effect in the berries, this will effect on the human healthy, also whiteness of berries by effect on the pigments (Anthocyanin or Chlorophyll) as a result of $SO_2$ fumigation and this effect on berries appearance and test, which considered the most important factors of quality (Yahia *et al.,* 1983 and lichter *et al.,* 2002). In addition to that, Gao *et al.*, (2003) demonstrated the sensitivity effect of table grape cultivars to $SO_2$ at room and at low temperature. However, the relationship between bleaching index, fumigation concentration and exposure time was not liner. Also, the bleaching index was strongly correlated with fumigation concentration and exposure time. Furthermore, the effect of fumigation concentration on bleaching was more significant than the effect of exposure time. i.e. bleaching increased with increase fumigation concentration.

Ethanol is common food additive with potent antimicrobial activity (Larson and Morton, 1991). Ethanol dips and vapors have been reported to control postharvest diseases of peaches, citrus fruit, and table grapes (Larsonand Morton, 1991; Feliciano et al., 1992; Smilanick *et al.*, 1995; Gabler and Smilanick, 2001; Gabler *et al.,*2002; Karabulut *et al.*, 2003), especially when heated (Smilanick et al., 1995; Margosan et al., 1997; Karabulut et al., 2004).

Polyphenols are the most important constituents of grapes from the point of view of functional properties, with catechins, flavonol glycosides, phenolic acids, stilbenes and anthocyanins all having been detected (Threlfall *et al.,* 2005). However,changes in anthocyanin profiles and loss of other phenolic compounds have been reported during advanced stages of ripening (Canals *et al.,* 2005).

The varietal difference in the color of black and red grapes results from the accumulation of anthocyanidins that are modified by the attachment of glucose moieties to form anthocyanins. Anthocyanins are generally limited to the vacuoles of a few cell layers below the epidermis that affect light scattering or penetration of violet-black or red skin tissue of grape cultivars (Shiraishi *et al.,* 2007). In addition to that, *V. vinifera* cultivars produce 3-monoglucoside and 3-p-coumarylglucoside derivatives of aglycones

based on the structural difference in the B-ring as follows: cyanidin, peonidin, delphinidin, petunidin, and malvidin (Fig. 1).

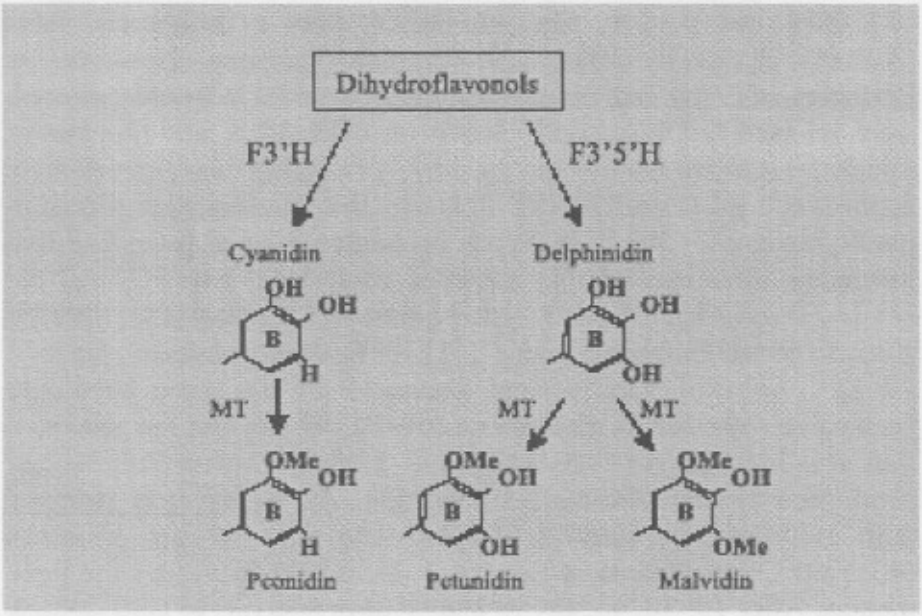


**Fig. 1. The B-ring of the anthocyanin biosynthetic pathway with modification of Boss *et al.* (1996a). Multi-step enzymatic contributions exist in the pathway from dihydroflavonols to cyanidin or delphinidin. F3'H: flavonoid 3'-hydroxylase, F3'5'H: flavonoid 3',5'-hydroxylase, MT: methyltransferase.**

The aim of this study was to use ethanol fumigation at two concentrations 75 and 100 % ((8 ml ethanol $kg^{-1}$), to demonstrate their effect comparison to sulfur pads and control, on total phenolic compounds, anthocyaninin and respiration rate, also to limit their effect on whole quantity and quality of color in "Helwany" grape berries and clusters, during cold storage, in purpose to enhanced table grape marketing, and to satisfied consumer demand.

# MATERIALS AND METHODS

## 1. Plant material

Table grape *Vitis vinifera* L. cv. "Helwany" was harvested at 17.1 Brix° and 0.30 % titratable acidity from a commercial farm (Alkafer- Syria) in 2008-2009 successive seasons. However, in laboratory of Pome and Vine Research Department in Sweida, clusters were selected homogeneously based on color, size, and absence of injuries, greenish rachis and healthy. Pr-cooling was immediately applied at 0 ± 1 C° and 90-95% RH, after that, clusters were packed in plastic boxes (40×30×10 cm) each containing 5 kg of fruit. And five treatments were applied: (1) untreated control, (2) one $SO_2$ pad per box (5 g $Na_2S_2O_5$), (3) one $SO_2$ pad per box (7.5 g $Na_2S_2O_5$), (4) 75% absolute ethanol vapors 8 ml $kg^{-1}$, (5) 100% absolute ethanol vapors 8 ml $kg^{-1}$, (ethanol vapors were generated by air pump tube into fumigation chamber its dimensions 100×75×90 cm, and the generated time was 30 mints for every treatment). Each box was a replicate and there were three replicates per treatment. All boxes were wrapped with individual permeability polyethylene bags 40 μm thickness (40×60cm), and then stored at 0 ± 1 C° and 90-95 % RH for three months. After 15, 30, 45, 60, 75 and 90 days intervals of cold storage, boxed grapes from each treatment were removed from cold storage, and fruit anthocyanin pigment, total phenolic compounds and respiration rate were evaluated.

## 2. Anthocynin determination

Anthocyanin was extracted using 2 g of berries skin tissues homogenized in 20 ml ethanol and 1% HCL at ratio 85:15 v/v respectively according to (Hurst, 2002). The extraction washed and filtered with the solvent 3 times until the grind berries skin in filter paper became colorless, then absorbance of anthocyanin measured at wave length 525 nm , by spectrophotometer (Hitachi U- 29000-Japan) and the concentration was calculated by formula deepening on Beer and Lambert:

$$A = a.\ b.\ c$$

A: absorbance at wave length 525.
a: Molar extinction coefficient ($L.cm^{-1}.mol^{-1}$).
b: cuvette cell length (cm).
c: concentration of anthocyanin (mg 100 $g^{-1}$).

anthocyanin (mg.100g$^{-1}$) was measured as Malvadin 3-glucoside according to (Boss *et al.*, 1996a).

**3. Total phenolec compounds**

10 g of fresh berries or clusters pedicels were homogenized by mortar with 30 ml absolute ethanol according to (Wada and Ou, 2002). The sample was extracted in ultrasonic bath for 45 minutes. Then the samples were centrifuged for 7 minutes at 3000 rpm. The supernatant was filtered through paper filter, transferred into vial prior analyses.

Total phenolic content was assessed by using the Folin-Ciocalteau phenol reagent method (Singleton and Rossi, 1965). To 2ml of the samples, 3 ml of bidestilated water and 200 µl of Folin-Ciocalteau reagent were added, in 10 ml volumetric flask After 30 sec to 8 min, 4ml of sodium carbonate (7 % w/v) was added. The extracts were stand for 2 hr. at room temperature. On the basis of measured absorbance at 750 nm, determination from calibration curve and considering dilutions, the total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per 100 gram fresh sample.

**4. Respiration rate measurement**

Respiration rate was recorded by carbon dioxide produced from grape berries after harvest and than every 15 days of cold storage by titration of NaOH 0.1 N against HCL 0.1 N with phenol phathaline indicator (A.O.A.C. 1970).

**5. Statistical analysis**

Mean comparisons were made using Duncan's multiple range tests at 5% probability (Duncan, 1955), by MSTATC – ANOVA.

## RESULTS AND DISCUSSION

**1. Effect on anthocyanine content (mg-100 g$^{-1}$)**

The application of ethanol at two concentrations was great effect on fruit quality of "Helwany" grape cultivar during two successive season's 2008 & 2009.However, a line graph (1, A) and table (1) shows that, anthocyanin concentration in the berries skin increased sharply by application of 75% ethanol and reached a peak after 75 days of cold storage which was 24 mg. 100 g$^{-1}$ fresh skin berries, also the anthocyanin concentration increased steadily at 100 % ethanol

treatment during the storage periods, but the concentration of anthocyanin decreased gradually by increasing the storage time, and as example by application of treatment (3), they were 8.5, 7.5, 6.5, 5.4, 4.4 and 2.9 mg. 100 $g^{-1}$ respectively after 15, 30, 45, 60, 75 and 90 days of cold storage. In addition to that, significant differences were found between the application on 75 % of ethanol comparing to other treatments in all periods of storage, except after 15 days of cold storage the significant deference between 75% and 100 % ethanol was absence. Also, the figure illustrates that, the lowest concentration of anthocyanin was recorded in control after 75 and 90 days of cold storage time. Whereas, the application of treatment (2) and (3) badly affected on anthocyanin content and after 45 days of storage the lowest value was noticed by the treatment (3). These results agreed with (Crisosto *et al.,* 2002) who demonstrated that, the necessary concentration of (SO2), may induce injuries in both rachis and berries. Also, SO2 can cause unacceptable bleaching injuries to berries (Crisosto and Mitchell, 2002), and organoleptic quality may be compromised as well (Chervin *et al.,* 2005). The changes in the content of anthocyanin return to continues synthesis of nthocyanin after harvest and also in during cold storage (Kalt *et al.*, 1999). Other studies have shown that ethanol increase the concentration of anthocyanin and related synthesis pathway (El Kereamy *et al.,* 2002), and flavonoid 3-*O*- glucosyltransferase (UFGT) genes play an important role in increasing the accumulation of anthocyanin in red cultivars, as well as its role in determining the difference between red and white grapes (Boss *et al.,* 1996b; Kobayashi *et al.,* 2001).

**2. Effect on phenolic compounds**

In the other hand, Figure (1, B) and table (2) declares that, total phenolic compounds in berries were differently changed according to the treatment applied in all periods of cold storage. The content of phenolic compounds increased gradually in control and sulfur pads (3) treatments, and reached a peak after 60 days of cold storage, whereas, the application of (2) treatment rose the phenolic contents dramatically until 75 days of cold storage, after that the concentration of phenolic in berries fell slightly until the end of storage time. In the other hand, total phenol;ic compounds in berries increased by application of (4) and (5) treatments, and reached a peak after 45 days of cold storage, which reached 4.1 (mg. 100 $g^{-1}$) at two concentrations.

However, the application of ethanol at two concentrations has greatly effect on the total phenolic compounds in berries, and significant differences were found comparing to sulfur pads and control, for instance after 60 days of cold storage total phenolic compounds were 3.2, 3.7, 3.7, 3.9, 3.9 mg /100 $g^{-1}$ for control, 2, 3, 4, 5 treatments respectively with significant variation between the same kind of treatment. But, the highest concentration of phenolic compounds was recorded for sulfur dioxide pads in (2) and (3) treatments after 75 and 90 days intervals, with significant differences between all treatments. This data also illustrated that, the lowest level of phenolic compounds in berries was noticed by control in all periods of cold storage. Also, the line graph (2, A) and table (2) shows that, total phenolic compounds in pedicels were affected by the time of storage and varied according the sort of treatment

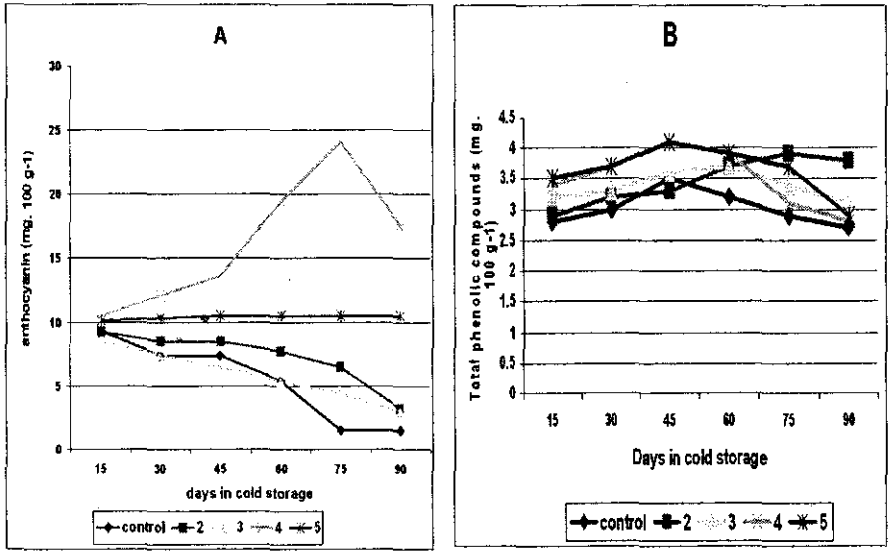


**Fig.(1): Effect of Ethanol and Sulfur dioxide fumigation on anthocyanin (mg. 100 $g^{-1}$) in berries skin and Total phenolic compounds (mg. 100 $g^{-1}$) in berries during cold storage of "Helwany" grape cultivar, at 0-(-1) C° and 90-95 % RH, in 2008-2009 successive seasons.**

**Table (1): Effect of Ethanol and Sulfur dioxide fumigation on anthocyanin (mg. 100 $g^{-1}$) in berries skin and Total phenolic compounds (mg. 100 $g^{-1}$) in berries during cold storage of "Helwany" grape cultivar, at 0-(-1) C° and 90- 95 % RH, in 2008-2009 successive seasons.**

| Treatment | Days in Cold Storage | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Anthocyanin (mg. 100 $g^{-1}$) | | | | | | Total phenolic compounds (mg. 100 $g^{-1}$) | | | | | |
| | 15 | 30 | 45 | 60 | 75 | 90 | 15 | 30 | 45 | 60 | 75 | 90 |
| 1 | 9.3 B* | 7.4 D | 7.4 D | 5.4 D | 1.5 D | 1.5 D | 2.8 E* | 3 D | 3.5 C | 3.2 C | 2.9 E | 2.7 D |
| 2 | 9.2 B | 8.5 C | 8.5 C | 7.7 C | 6.5 C | 3.2 C | 2.9 D | 3.2 C | 3.3 D | 3.7 B | 3.9 A | 3.8 A |
| 3 | 8.5 C | 7.5 D | 6.5 E | 5.4 D | 4.4 C | 2.9 C | 3.2 C | 3.3 B | 3.6 B | 3.7 AB | 3.4 C | 3.1 B |
| 4 | 10.5 A | 12.1 A | 13.6 A | 19.4 A | 24 A | 17.5 A | 3.4 B | 3.7 A | 4.1 A | 3.9 A | 3.1 D | 2.8 D |
| 5 | 10.1 A | 10.3 B | 10.5 B | 10.5 B | 10.5 B | 10.5 B | 3.5 A | 3.7 A | 4.1 A | 3.9 A | 3.7 B | 2.9 C |

*** Means having the same letter (s) in the same column are not significant at a 5 % level.**

**** Treatment 1= Untreated control & 2= 5g $Na_2S_2O_5$/box & 3= 7.5 g $Na_2S_2O_5$/box**

**4=75% absolute ethanol vapors & 5= 100% absolute ethanol vapors**

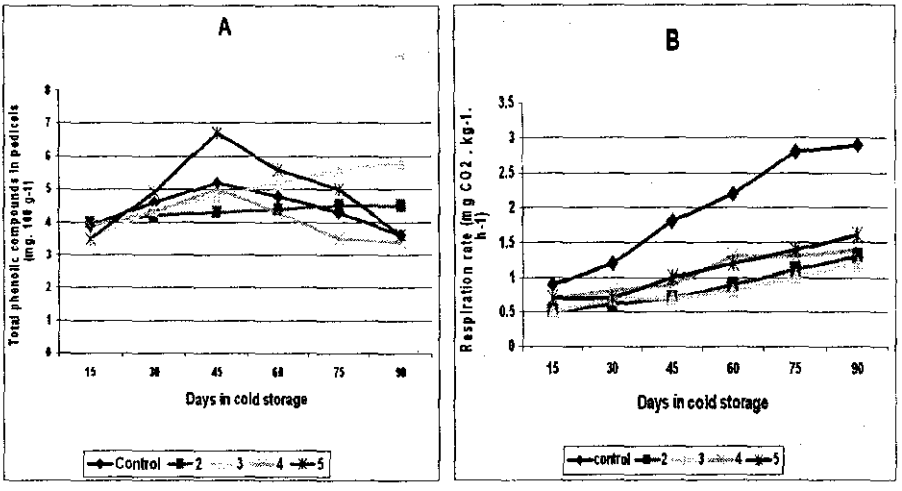


**Fig. (2): Effect of Ethanol and Sulfur dioxide fumigation on Total phenolic compounds in pedicels (mg. 100 $g^{-1}$) and Respiration rate (mg $CO_2$ . $kg^{-1}$. $h^{-1}$) during cold storage of "Helwany" grape cultivar, at 0-(-1) C° and 90- 95 % RH, in 2008-2009 successive seasons.**

The application of sulfur dioxide pads at two concentrations gave the same trend in all storage periods, and the content of phenolic compounds increased gradually, and reached a peak after 75 days of cold storage which were 4.5, 5.6 for treatment 2 and 3 respectively. Whereas, the control and ethanol treatment slightly increased the total phenolic compounds in pedicels and reached the top after 60 days of cold storage, after that the phenolic contents decreased gradually until the end of storage time. In the other hand, significant differences were found between all treatments in all storage periods and this variation changed by prolonged of storage time, for example after 15 days of cold storage the highest level for phenolic compounds was 4 mg. 100 $g^{-1}$ by applied of treatment (2) with significant differences comparing to other treatments. But the level of total phenolic compounds was changed after 30, 45 and 60 days of cold storage, and the treatment with 100 % ethanol occupied the highest concentration, with 4.9, 6.7 and 5.6 (mg. 100 $g^{-1}$) respectively with significant variation comparing to other treatments. In addition to that, the changes in total phenolic remained in fluctuated, which gave 5.6 and 5.8 (mg. 100 $g^{-1}$)

**Table (2): Effect of Ethanol and Sulfur dioxide fumigation on Total phenolic compounds in pedicels (mg. 100 $g^{-1}$) and Respiration rate (mg $CO_2$ . $kg^{-1}$. $h^{-1}$) during cold storage of "Helwany" grape cultivar, at 0-(-1) C° and 90- 95 % RH, in 2008-2009 successive seasons.**

| Treatment | Days in cold storage | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total phenolic compounds in pedicels (mg. 100 $g^{-1}$) | | | | | | | Respiration rate (mg $CO_2$ . $kg^{-1}$. $h^{-1}$) | | | | | | |
| | 0 | 15 | 30 | 45 | 60 | 75 | 90 | 0 | 15 | 30 | 45 | 60 | 75 | 90 |
| 1 | | 3.9 AB | 4.6 B | 5.2 B | 4.8 C | 4.3 D | 3.6 C | | 0.9 A | 1.2 A | 1.8 A | 2.2 A | 2.8 A | 2.9 A |
| 2 | | 4 A | 4.2 D | 4.3 E | 4.4 D | 4.5 C | 4.5 B | | 0.5 B | 0.6 B | 0.7 B | 0.9 BC | 1.1 B | 1.3 B |
| 3 | | 3.5 C | 4.4 C | 4.8 D | 5.2 B | 5.6 A | 5.8 A | | 0.5 B | 0.7 B | 0.7 B | 0.8 C | 1 B | 1.2 B |
| 4 | | 3.9 B | 4.3 C | 5 C | 4.3 E | 3.5 E | 3.4 D | | 0.7 AB | 0.8 B | 0.9 B | 1.3 B | 1.2 B | 1.4 B |
| 5 | | 3.5 C | 4.9 A | 6.7 A | 5.6 A | 5 B | 3.6 C | | 0.7 AB | 0.6 B | 1 B | 1.2 BC | 1.4 B | 1.6 B |

* Means having the same letter (s) in the same column are not significant at a 5 % level.

** Treatment 1= Untreated control & 2= 5g $Na_2S_2O_5$/box & 3= 7.5 g $Na_2S_2O_5$/box

4=75% absolute ethanol vapors & 5= 100% absolute ethanol vapors

respectively after 75 and 90 days of cold storage by application of treatment (3), with significant differences between all treatments, and this trend was noticed in two average of successive seasons (2008-2009). in this work the loss of quality during storage was accompanied of the functional properties, in control grapes (pedicels and berries) total phenolic compounds were fluctuated during cold storage, and could be responsible for the reduction of total antioxidant activity ( Valero *et al.,* 2006). In contrast, the applied of sulfur pads and ethanol fumigation led to delay in the loss of total phenolics, and the proportion of total phenolics were correlated with increases of total antioxidant activity in pedicels and berries. In other table grape cultivars total antioxidant activity has also been correlated with phenolic compounds (Davalos *et al.*, 2005), these polyphenols being postulated for use as natural antioxidants in foods (Bonilla *et al.,* 1999; Pazos *et al.,* 2005). It was also suggested that ethanol vapours might have reduced polyphenol oxidase activity, which is known to be involved in the blackening of protea leaves (Whitehead and de Swardt, 1982), also (Pesis, 2005) illustrated that, Ethanol may also be able to inhibit other disorders, which involve oxidative activity.

**2. Effect on respiration rate (mg CO2. kg-1. h-1)**

Figure (2, B) and table (2) illustrated that; respiration rate (mg $CO_2$. $kg^{-1}$. $h^{-1}$) was increased by prolonged of storage life, in all periods and by application of all treatments. The line rose sharply by control and reached highest level after 90 days of cold storage, whereas the application of sulfur pads and ethanol induced steadily increase in respiration rate during all periods of storage. In addition to that, different variations were found between all treatments and control in all storage durations, except after 60 days of cold storage, whereas the lowest respiration rate occurred by applied treatment (3) with significant differences comparing to other treatments, and the values was for example 2.2, 0.9, 0.8, 1.3 and 1.2 (mg $CO_2$. $kg^{-1}$. $h^{-1}$) for treatment (1), (2), (3), (4) and (5) respectively, and the same trend was noticed in two successive seasons. In this field many researchers demonstrated that, postharvest application of Ethanol and acetaldehyde to non-climacteric fruit has been reported to cause induction of CO2 production (a climacteric-like respiration) in orange (Fidler, 1968; Pesis and Avissar, 1989), fig (Hirai *et al.,* 1968), strawberry and blueberry (Janes *et al.*, 1978), and grape (Pesis and

Mariniansky, 1992). In fig and orange, acetaldehyde application led to reduced acidity (Hirai *et al.,* 1968; Pesis and Avissar, 1989).

In conclusion, the application of ethanol at two concentrations, improved fruit quality, through enhancing fruit color, increasing total antioxidant activity and reducing respiration rate during cold storage in "Helwany" grape cultivar, Additional research is still required to study gene expression for postharvest ethanol application, in accumulation of anthocyanin and phenolic compounds in berries of "Helwany" cultivar during cold storage.

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# تأثير التبخير بالإيثانول على صبغة الأنثوسيانين و المواد الفينولية الكلية لثمار العنب صنف الحلواني خلال التخزين المبرد

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تم تبخير ثمار العنب صنف الحلواني بتركيزين 75 و 100 % من الإيثانول المطلق ( 8 مل/ كغ ثمار)، كما عوملت الثمار بشرائح ميتا بيسلفيت الصوديوم (1غ و 1.5 غ/ كغ ثمار) في أكياس من البولي إيثلين سماكة 40 ميكرون، وخزنت الثمار بدرجة 0 ± 1 م° و رطوبة نسبية 90–95 % لمدة ثلاثة شهور، في وحدات التبريد التابعة لقسم بحوث التفاحيات والكرمة بالسويداء خلال موسمين متتاليين 2009/2008.حيث حسنت المعاملة بالإيثانول محتوى صبغة الأنثوسيانين ( ملغ/ 100 غ) في قشرة حبات العنب. وكان محتوى المواد الفينولية الكلية( ملغ/ 100 غ) في الحبات و العناقيد أعلى مقارنة مع الشاهد، بينما انخفض محتوى المواد الفينولية الكلية ( ملغ/ 100 غ) و معدل تنفس العناقيد (ملغ $CO_2$/ كغ ثمار/ سا) مقارنة مع تطبيق شرائح الكبريت في كلا الموسمين.

**الكلمات المفتاحية:** العنب، صبغة الأنثوسيانين، المواد الفينولية، معدل التنفس، التخزين المبرد.