AcademicPres

Available online at www.notulaebiologicae.ro

Print ISSN 2067-3205; Electronic 2067-3264

Not Sci Biol, 2012, 4(3):79-85

Notulae Scientia Biologicae

Effects of *Thymus vulgaris* Essential Oil on Decay Resistance and Quality of Iranian Table Grape

Mahsa GERANSAYEH[1], Younes MOSTOFI[2], Vahid ABDOSSI[1]

[1]*Azad University of Tehran, Science and Research Branch, Tehran, Iran; m.geransayeh@yahoo.com (*corresponding author)*

[2]*University College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran*

Abstract

Because of greater consumer awareness and concern regarding synthetic chemical additives, foods preserved with natural additives have become popular. Medicinal plants have been used by human being since ages in traditional medicine due to their therapeutic potential and the search on medicinal plants have led the discovery of novel drug candidates used against diverse diseases. Therefore *Thymus vulgaris* essential oil was applied in 'Bidaneh Qermez' grape cultivar at six concentrations (0, 100, 200, 300, 400 and 500 µl/l) in water. Quality characteristics (pH, decay, vitamin C, reducing sugars, weight loss, berry abscission, dehydration of rachis, berry cracking and sensory analyses) were evaluated. The results showed that treated fruits with essential oil had lower decay percentage, dehydration of rachis, berry abscission, berry cracking and higher pH, reducing sugars and storage quality compared to control. As a general result, essential oil treatment caused lower decay incident and longer storability.

Keywords: essential oil, *Lamiaceae*, perishable fruit, postharvest attributes, shelf-life

Introduction

Because of greater consumer awareness and concern regarding synthetic chemical additives, foods preserved with natural additives have become popular. This has led researchers and food processors to look for natural food additives with a broad spectrum of antimicrobial activity (Marino *et al.*, 1999). Recently there has been considerable interest in generally recognized as safe (GRAS) compounds. Plant essential oils are an example of GRAS compounds (Jobling, 2000). Essential oils are extracted from various aromatic plants. They are liquid, volatile, limpid, and rarely colored, lipid soluble and soluble in organic solvents with a generally lower density than that of water (Bakkali *et al.*, 2008). Some plant essential oils have been reported to have antimicrobial activity against a wide range of spoilage and pathogenic bacteria (Kivang *et al.*, 1991) and often have, in common, active phenolic groups (Holley and Patel, 2005). The efficacy of essential oil from clove and cinnamon against 6 fungi causing postharvest decay of grapes were studied. The best concentration to inhibit growth of 6 fungi was 400 mg/ml (Sukata *et al.*, 2008). A total of 14 odoriferous angiospermic essential oils were tested against the toxigenic strain of *Aspergillus flavus* and *Botrytis cinerea*. The essential oil of *Thymus vulgaris* L. showed highest antifungal efficacy (Kumar *et al.*, 2008). Microbial spoilage counts and losses of quality in term of sensory, nutritional and functional properties were significantly reduced in packages with added eugenol or thymol in table grapes (Valero *et al.*, 2006). In this study the essential oil of *Thymus vulgaris* L. has been investigated regarding its potential to inhibit pathogens production and safety for humans. In addition, the effects of *Thymus vulgaris* on quality-related attributes of table grapes were evaluated.

Materials and methods

Plant material

Table grapes (*Vitis vinifera* L.), cv. 'Bidaneh Qermez' were harvested randomly from a commercial vineyard located in Takestan, Qazvin, Iran at commercial maturity and transported to the laboratory. Grape clusters were divided into small clusters of approximately 300 g each, those with defects were discarded.

Essential oil treatment

Treatments with *Thymus vulgaris* essential oil (purchased from Zardband company, Tehran, Iran), were performed by dissolving the requisite amounts of *Thymus vulgaris* (0, 100, 200, 300, 400 and 500 µl/l) in 25 ml of 0.05 % tween-80 and then mixing with 475 ml of water. Grapes were dipped in the solutions for 1 min at room temperature and air dried (Hadizadeh *et al.*, 2009). Each treatment was replicated three times with 300 g fruits per replicate. All packages were stored at 4°C and 75 % RH in darkness for 75 days. Measurements were made at room temperature every 15 days.

Received 21 June 2012; accepted 30 September 2012

Gas Chromatography-Mass Spectrometry analysis

The analysis of the volatile constituents were run on a Heweltt-Packard GC/MS system (GC: 6890; MS: 5973). The fused- silica hp INNOWAX capillary column (30 m × 0.25 mm ID, film tickness of 0.32 μm) was directly coupled to the MS. The carrier gas was helium, with a flow rate of 1 mm/min. Oven temperature was programmed (60°C for 3 min, then 60-220°C at 5°C /min) and subsequently, held isothermal for 2 min. Injector temperature: 250°C, detector temperature: 300°C. Split ratio 1:20. Volume injected: 0.1 μl of 1% solution (diluted in hexane). The mass spectrometer was hp recording at 70 eV; scan time 1.5 sec; mass range 40-300 amu.

Identification of components

The components of the oil were identified by comparison of their mass spectra with those of a computer library (Wiley 275 library). Retention indices were calculated using retention times of n-alkanes that have been injected to the same instrument (Adams, 1995; Shibamoto, 1987).

pH rate

pH was measured using a pH meter Metrohm Lab 827 (Saini *et al.*, 2006).

Weight loss percentage

The effect of essential oil exposure on table grape weight loss was also investigated. Weight loss of individual bunches was recorded on the day of harvesting and after the different sampling dates expressed as percentage loss of original weight (Valero *et al.*, 2006).

Color changes

Color changes of berries were analyzed on the skin surface with a Minolta Chromameter CR400. Color was measured at the further two points apart on the equator of each berry and expressed as Chroma (Chroma = $[(a^2+b^2)^{0.5}]$) and Hue angle ($H° = \tan^{-1}(b^*/a^*)$) (Valero *et al.*, 2006).

Vitamin C content

The content of vitamin C was determined using indophenol procedure. 10 ml of samples were filtrated and titrated against sodium 2, 6-dichlorophenol indophenol dye to a faint pink color which persisted for 5-10 seconds. It was expressed as mg vitamin C/100g fruit weight (Titer x dye equiv. x dilution x100/ Wt. of sample) (Saini *et al.*, 2006).

Reducing sugars content

Reducing sugar was measured with the procedure Lane-Eynon. Mixed Fehling's solution was made by transferring 5 ml of solution A and 5 ml of solution B to a 250 ml conical flask and mixed well. Diluted fruit juice was added to mix Fehling's solution drop by drop. It was heated over Bunsen burner and boiled for two minutes. Then added three drops of methylene blue indicator (Saini *et al.*, 2006).

Decay percentage

Percent of decay was scored on a 1-9 scale, where: 0= intact fruit, 1= less than 10% Decay, 2= between 10-20% decay, 3= between 20-30% decay, 4= between 30-40% decay, 5= between 40-50% decay, 6=between 50-65% decay, 7= between 65-80% decay, 8= more than 80% decay (Nigro *et al.*, 2000).

Berry abscission, dehydration of rachis and berry cracking

Berries abscission was scored on a 1-5 scale, where: 1= very low, 2= low, 3= moderate, 4= high, 5= very high. For the rachis, symptoms of dehydration and browning for primary and secondary branches were evaluated on a ranked scale of 1-5, where= 1= green and fresh, 2= green, 3= semi-dry, 4= 50% dry, 5= absolutely dry (Valero *et al.*, 2006; Xu *et al.*, 2007). Number of cracked berries in 300 g was evaluated (Xu *et al.*, 2007).

Sensory evaluation

Sensory analyses to compare the quality of treated and control table grapes were carried out by a 10 trained adults aged 25-40 years. It was about aroma, taste, firmness, appearance and texture. Panelists scored grapes between 1-10. 10 being the best total quality and 1 being the worst (Martinez-Romero *et al.*, 2007). Assessments were continued until fruits condition were considered unacceptable.

Statistical analysis

Statistical analysis of the data obtained in the present study was carried out using split factorial method in a completely randomized design layout with 3 replications. Data obtained were subjected to analysis of variance (ANOVA). Means were separated by SNK and LSD test.

Results and discussion

Chemical composition of the essential oil was reported in Tab. 1, with their retention indices (RI), and the percentages of compounds.

pH rate

While time passed, in storage period, pH rate of grape juice increased (Tab. 2). Results showed that a significant increase in pH rate was experienced in samples which were treated with essential oil compared to controls (Fig. 1). pH of the grape extract is determined by changes in the amount of tartaric acid, it is expected that a relationship exists between the level of acidity and pH. When the level of acidity cuts down, pH of fruits extract increase. pH levels of strawberries and tomatoes with eucalyptus and cinnamon essential oils didn't change during storage period (Tzortzakis, 2007).

Tab. 1. Chemical composition of the *T. vulgaris* essential oil

Chemical composition	Percentages of compounds	Retention time	Retention indices
Alpha-pinene	1.53	10.45	939
Camphene	0.21	10.90	954
Beta-pinene	5.24	11.63	979
Myrcene	0.98	11.73	991
Alpha-phellandren	0.2	12.25	1003
Alpha-Terpinene	1.34	12.54	1017
Para cymen	19.79	12.80	1025
Limonene	0.92	12.88	1029
Beta-phellandrene	0.53	12.94	1030
Gamma-Terpinene	20.28	13.64	1060
Terpinolene	0.26	15.36	1076
Para-menthatriene	0.1	15.48	1110
Estragole	1.23	16.80	1179
Bornyl formate	0.13	17.62	1289
Thymol	48.28	18.50	1290
Carvacrol	2.54	18.92	1299
Hexa decanoic	0.43	28.11	1600

of essential oil resulted in an increase in decay resistance of samples (Fig. 2). The samples which were treated with concentration 500 µl/l endured more than other groups, up to the 75 days. These samples did not show any sign of decay up to the 75 days. The use of 1 µl/ml *Thymus vulgaris* essential oil could significantly enhanced plant resistance to *Botrytis cinerea* (Kumar *et al.*, 2008). Higher concentrations of *Thymus vulgaris* essential oil led to greatest control of bacteria (Celikel and Kavas, 2008). Lemongrass essential oil could significantly control *B. cinerea*. Higher concentration of essential oil led to the greatest decay control (Tzortzakis and Economakis, 2007). Essential oils antimicrobial activity related to their hydrophobic property and they have high antioxidant role within the plants (Brul and Coote, 1999).

Vitamin C

Vitamin C content of grapes decreased significantly along the storage (Tab. 2). The samples which were treated with 300 and 400 µl/l essential oil had the lowest vitamin

Tab. 2. The mean of evaluated parameters during the storage period in table grape cv. 'Bidaneh Qermez'

Time (Day)	pH	Decay (%)	Vit. C (mg/100 g fruit weight)	Reducing sugars (%)	Weight loss (%)	Hue angle	Chroma	Berry abscission	Rachis dehydration	Berry cracking (g)	Fruit quality
0	3.55d	0.0e	15a	13.92e	0.00e	46.1a	9.77a	0.00e	1.00e	0.0c	10a
15	3.63c	0.5d	13b	14.33d	0.10d	41.4b	9.21b	2.11d	2.50d	0.0c	7.53b
30	3.74bc	1.7c	10c	15.02c	0.14c	40.2bc	7.97c	2.89cd	3.22c	334b	7.50b
45	3.79b	1.7c	10c	15.02c	0.14c	40.5bc	7.02d	3.27c	4.33b	432a	
60	3.85a	4.0b	9.6cd	15.71b	0.21b	39.0c	6.74de	4.00b	4.89a	430a	
75	3.91a	7.3a	8d	17.04a	0.23a	38.9c	6.50e	4.67a	4.98a	437a	

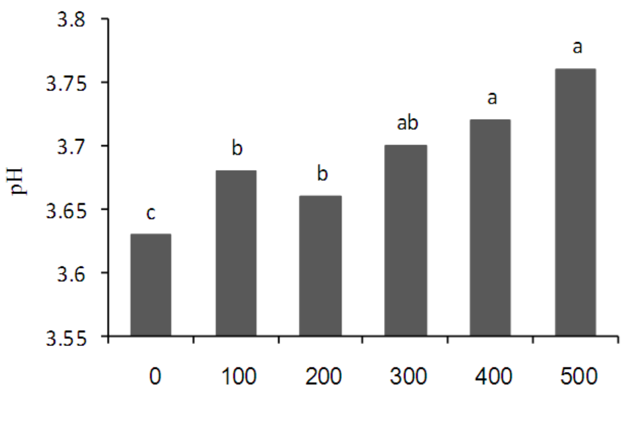

Fig. 1. The effect of essential oil treatment on pH of 'Bidaneh Qermez' table grape

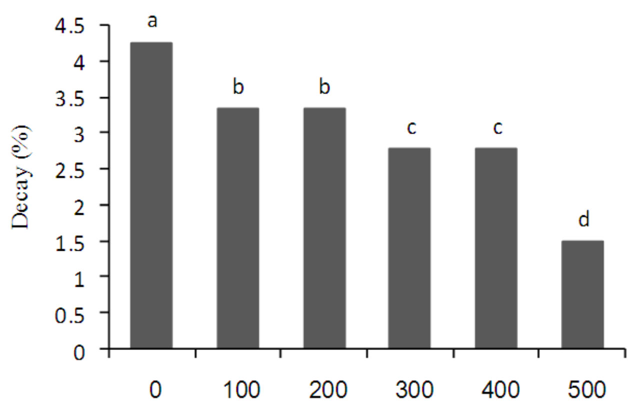

Fig. 2. The effect of essential oil treatment on decay percentage of 'Bidaneh Qermez' table grape

Decay percentage

During the storage period, the amount of decay increased, so that in the 75th day the rate of decay reached 7.33 % (Tab. 2). The controls with the mean of 4.28 % had the highest percent of decay. Increasing the concentration

C compared to others (Fig. 3). No difference was observed between the control and essential oil treated samples with concentration 100, 200 and 500 µl/l in regard to vitamin C content. Vitamin C content of treated fruits with eugenol and thymol decreased along the storage. In treated samples

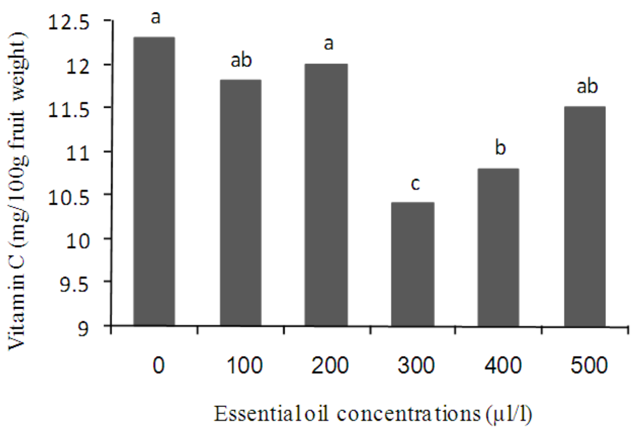

Fig. 3. The effect of essential oil treatment on vitamin C of 'Bidaneh Qermez' table grape

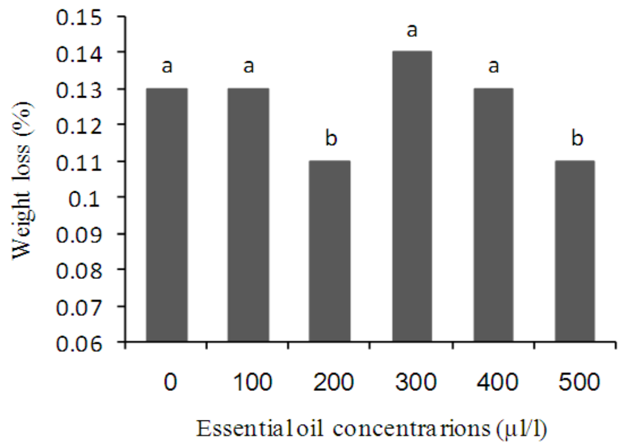

Fig. 5. The effect of essential oil treatment on weight loss of 'Bidaneh Qermez' table grape

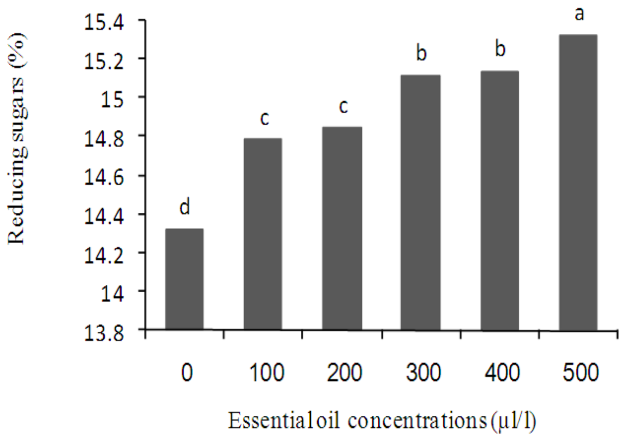

Fig. 4. The effect of essential oil treatment on reducing sugars of 'Bidaneh Qermez' table grape

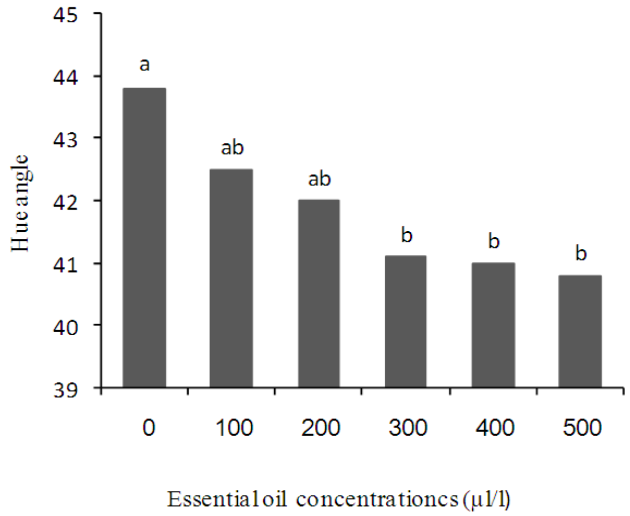

Fig. 6. The effect of essential oil treatment on hue angle of 'Bidaneh Qermez' table grape

more vitamin C was reduced compare to controls (Valero *et al.*, 2006). L-ascorbic acid (AA) is the principal biologically active form of vitamin C. Fruits show a gradual decrease in AA content as the storage duration increases (Adisa, 1986).

Reducing sugars content

While time passed, reducing sugars rate increased (Tab. 2). Results showed that increase in the concentration of essential oil, led to an increase in reducing sugars content (Fig. 4). Samples with 500 µl/l essential oil had the highest reducing sugars content compared to others. Reducing sugars in grapes which were treated with thymol and eugenol decreased significantly compared with controls. This finding was inconsistent with results obtained in experiment. Reducing sugars of kiwifruits increased gradually at first 7 days then decreased (Wang and Buta, 2003). When reducing sugars decrease, gray mold percent of grapes increase (Hill *et al.*, 1981). It can be concluded that the increase in reducing sugars can enhance plant resistance against pathogens, because it can affect antioxidant systems of fruit (Rolland *et al.*, 2006).

Weight loss percentage

Weight loss percentage of fruits increased in the storage period gradually (Tab. 2). There was no significant difference among different concentrations of essential oil and controls in regard to weight loss except samples treated with 200 and 400 µl/l essential oil (Fig. 5). It seems that essential oil did not affect weight loss of samples. The rate of weight loss of treated tomatoes and strawberries with essential oil and control was low and there was no significant difference between them (Tzortzakis, 2007). Weight loss of fruit occurs due to water loss and reduction in stored material via respiration.

Hue angle

The hue angle decreased along the storage (Tab. 2). The samples which were treated with 100 and 200 µl/l essential oil and controls had the highest hue angle compared to others (Fig. 6). Essential oil did not affect significantly color of treated strawberries and tomatoes (Tzortzakis, 2007). Treatments that reduce the rate of fruit ripening caused hue angle reduction (Thumula, 2006).

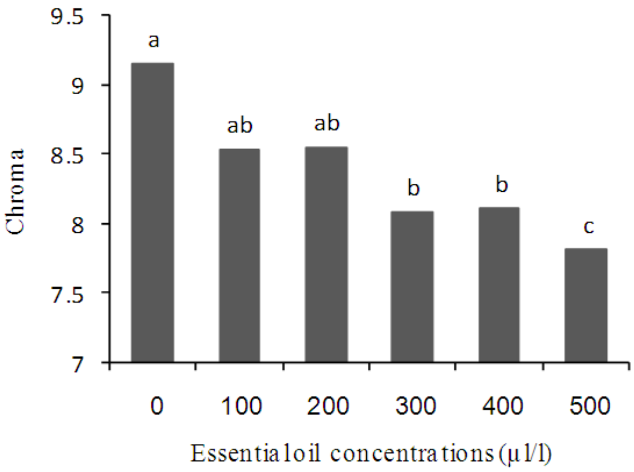

Fig. 7. The effect of essential oil treatment on chroma of 'Bidaneh Qermez' table grape

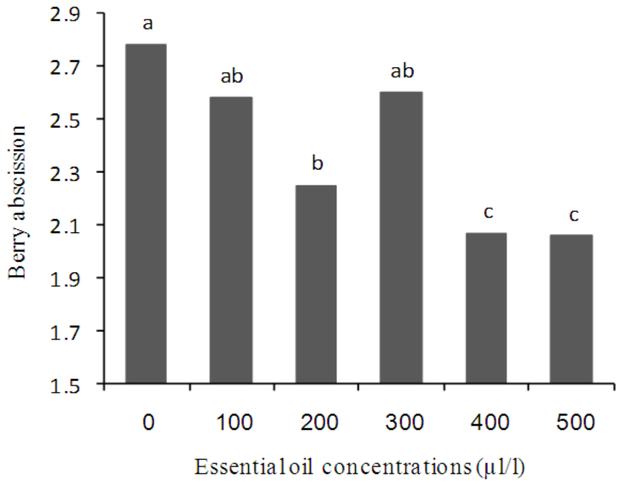

Fig. 8. The effect of essential oil treatment on berry abscission of 'Bidaneh Qermez' table grape

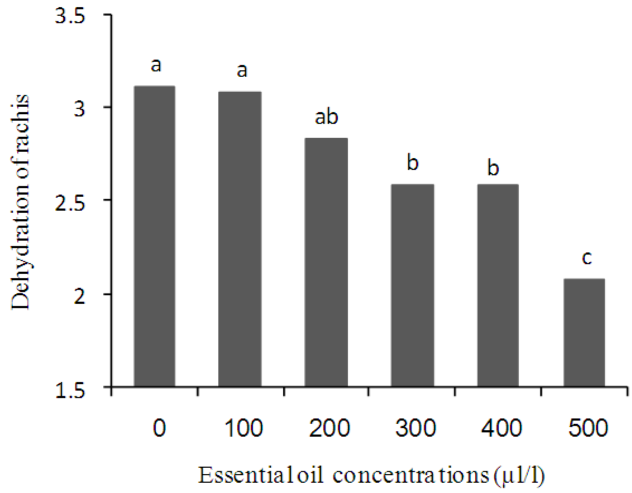

Fig. 9. The effect of essential oil treatment on dehydration of rachis of 'Bidaneh Qermez' table grape

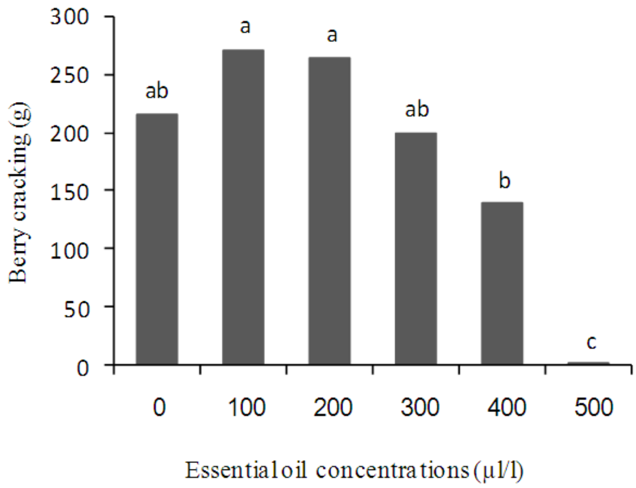

Fig. 10. The effect of essential oil treatment on berry cracking of 'Bidaneh Qermez' table grape

Chroma

The chroma reduced along the storage (Tab. 2). There was no significant difference among samples treated with 100 and 200 µl/l essential oil and controls (Fig. 7). Only the samples with concentration 500 µl/l showed a significant decreased in chroma rate with the mean of 7.81. When the samples experience weight loss and dehydration during the storage period, the phenomenon of browning berries occurs and cause more color changes in them. Reduction in the amount of green pigment over time is the main cause of chroma reduction (Artes-Hernandez and Aguayo, 2004).

Berry abscission

Results showed that there was a significant increase in berry abscission along the storage (Tab. 2). The maximum berry abscission was observed in controls, whilst the samples with concentrations 400 and 500 µl/l showed the lowest berry abscission (Fig. 8). ABA content in the pedicle with out berry was much higher than the pedicle with berry attached (You-Mei *et al.*, 2000). It seems that high concentration of essential oil may prevented production of ABA and resulted in lower berry abscission. Besides, reduction of respiration rate and water loss resulted in reduction in berry abscission in treated fruits.

Dehydration of rachis

Result showed that while time passed dehydration of rachis increased to 4.98 at the end of the storage period (Tab. 2). Treated samples with 500 µl/l concentration preserved their freshness of the rachis longer than other samples (Fig. 9). It appears that as time passed and as the rate of berry and rachis respiration increased, more water was removed from their tissues and dehydration of rachis appeared.

Berry cracking

During the storage period, the amount of cracked berries increased (Tab. 2). Treated samples with 500 µl/l concentration had the lowest berry cracking and there was no significant difference among control and treated samples

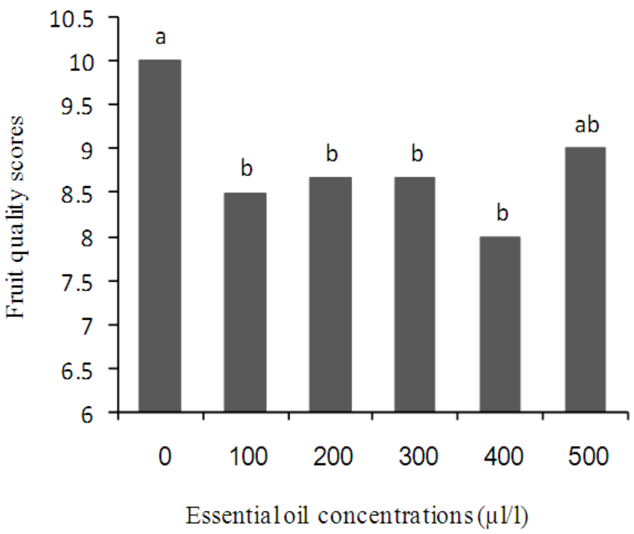

Fig. 11. The effect of essential oil treatment on fruit quality of 'Bidaneh Qermez' table grape

with concentration 100, 200 and 300 µl/l (Fig. 10). Because polyethylene package performs as a barrier against water steam it condenses the water vapor in the package atmosphere on the surface of grapes and leads to re-absorption of water. On the cool surface of the fruit due to high water absorption gaps appear in the cell walls that cause cracking of the berries skin (Considine and Kriedemann, 1972). It seems high concentrations of essential oil led to reduction in water loss.

Quality of fruits

The quality of fruits (color, texture, aroma, taste and appearance of fruits) reduced along the storage (Tab. 2). The control and treated samples with 100, 200 and 300 µl/l concentrations were infected in the first 30 days. Therefore, there was no data available after that. Quality of treated samples with 400 and 500 µl/l thyme essential oil evaluated until 45 days of storage. Treated samples preserved a better quality compared to controls (Fig. 11). There was no significant difference among different concentrations of essential oil in regard to quality. Except samples which treated with 400 µl/l essential oil. They showed a significant decreased in quality of fruits. Essential oil did not have any adverse effect on aroma and taste of treated tomatoes and strawberries (Tzortzakis, 2007). Grapes that treated with thymol and eugenol had better quality compared to controls (Valero *et al.*, 2006).

References

Adams RP (1995). Identification of essential oil components by Gas Chromatography/Mass Spectrometry. Allured Publishing Corporation, Carol Stream, Illinois.

Adisa VA (1986). The influence of molds and some storage factors on the ascorbic acid content of orange and pineapple fruits. Food Chem 22:139-146.

Artes-Hernandez F, Aguayo E (2004). Alternative atmosphere treatments for keeping quality of autumn seedless table grapes during long term cold storage. Postharvest Biol Technol 31:59-67.

Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008). Biological effects of essential oils-A review. Food Chem Toxicol 49:446-457.

Brul S, Coote P (1999). Preservative agents in foods made of action and microbial resistance mechanisms. Internat J Food Microbiol 50:1-17.

Celikel N, Kavas G (2008). Antimicrobial properties of some essential oils against some pathogenic microorganisms. Czech J Food Sci 26:174-181.

Considine JA, Kriedemann PE (1972). Fruit splitting in grapes: determination of the critical turgor pressure. Austral J Agr Res 23:17-24.

Hadizadeh I, Peivastegan B, Hamzeh Zarghani H (2009). Antifungal activity of essential oils from some medicinal plants of Iran against *Alternaria alternate*. Am J Appl Sci 6:857-861.

Hill GS, Kitleer F, Huth G, Schlosser E (1981). Resistance of grapes in different development stages of *Botrytis cinerea*. Phyto Pathol Zeitch 102:328-338.

Holley RA, Patel D (2005). Improvement in shelf life and safely of perishable foods by plant essential oils and smoke antimicrobials. Food Microbiol 22:273-292.

Jobling J (2000). Essential oils: A new idea for postharvest disease control. Good Fruit Vegetables Magaz 11:50.

Kivanc M, Akgul A, Dogan A (1991). Inhibitory and stimulatory effects of cumin, oregano and their essential oils on growth and acid production of *Lactobacillus plantarum* and Leuconostoc mesenteroides. Internat J Food Microbiol 13:81-86.

Kumar A, Shukla R, Singh P, Prasad CS, Dubey NK (2008). Assessment of *Thymus vulgaris* L. Essential oil as a safe botanical preservative against postharvest fungal infestation of food commodities. Internat Food Sci Em Technol 9:575-580.

Marino M, Bersani C, Comi G (1999). Antimicrobial activity of the essential oils of *Thymus vulgaris* L. measured using a bioimpedometric method. J Food Protect 62:1017-1023.

Martinez-Romero D, Guillen F, Valverde JM, Bailen G, Zapata P, Serrano M (2007). Influence of carvacrol on survival of *Botrytis cinerea* inoculated in table grapes. Internat J Food Microbiol 115:144-148.

Nigro F, Ippolito A, Lanttanzio V, Di Venere D, Salerno M (2000). Effect of ultraviolet-C light on postharvest decay of strawberry. J Plant Pathol 82:29-37.

Rolland F, Gonzalez E, Sheen J (2006). Sugar sensing and signaling in plants: Conserved and novel mechanisms. Ann Rev Plant Biol 57:675-709.

Saini RS, Sharma KD, Kaushik RA, Dhankhar OP (2006). Laboratory Manual Analytical Techniques in Horticulture.

Agrobios (India). IST Edition, 12-26 p.

Shibamoto T (1987). Retention indices in essential oil analysis in capillary Gas Chromatography, 259-275 p. In: Essential oil analysis. Sandra P, Bicchic A (Eds.). Alfred Heuthig-Verlag. New York.

Sukata V, Haruthaithanosan V, Chntarapanont W, Dilokkunanat V, Suppakul P (2008). Antifungol activity of clove and cinnamon oil and their synergistic against postharvest decay fungi of grape in vitro. Nat Sci 42:169-174.

Thumula P (2006). Studies on storage behavior of tomatoes coated with chitosan-lysozyme films. MSc Thesis. Faculty of Agricultural and Environmental Sciences. Mc Gill Univ Montreal, Quebec, Canada.

Tzortzakis NG (2007). Maintaining postharvest quality of fresh produce with volatile compounds. Internat Food Sci Em Technol 8:111-116.

Tzortzakis NG, Economakis CD (2007). Antifungal activity of lemon grass (*Cympopogon citrates* L.). Essential oil against key postharvest pathogens. Internat Food Sci Em Technol 8:253-258.

Valero D, Valverde JM, Martinez-Romero D, Guillen F, Catioo S, Serrano M (2006). The combination of modified atmosphere packaging with eugenol or thymol to maintain quality, Safety and functional properties of table grapes. Postharvest Biol Technol 41:317-327.

Wang CY, Buta JG (2003). Maintaining quality of fresh-cut kiwifruit with volatile compound. Postharvest Biol Technol 28:181-186.

Xu WT, Huang KI, Guo F, Qu W, Yang JJ, Liang ZH, Luo YB (2007). Postharvest grapefruit seed extract and chitosan treatments of table grapes to control *Botrytis cinerea*. Postharvest Biol Technol 46:86-94.

You-Mei WU, Jian-Chai REN, Xue-Zeng HUA, Yu LIU (2000). Postharvest berry abscission and storage of grape fruit. Acad Scinica 32 p.