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Effect of thymol treatment on decay, postharvest life and quality of strawberry (*Fragaria ananassa)* Fruit cv. 'Gaviota

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Key words: Thymol, Quality attributes, Storability, Decay, *In vivo*.

Abstract

Strawberry as a non-climacteric and perishable fruit that is susceptible to fungal infections especially *Botrytis cinerea*. Therefore, its storage life is too short. Alternatives to the use of conventional fungicides are needed because of concerns about human health risks. Postharvest application of conventional fungicides to fruits is prohibited. The aim of this study was evaluation of non-chemical and safe compounds like thymol to prolonged fruit storage life and maintained quality and quantity characteristics of strawberry fruit cv. Gaviota. Therefore, thymol at 4 concentration (0, 125, 250, 500 and 1000 $\mu l l^{-1}$) and 2 treatment methods (spray on fruits and paper disk method) in *in vivo* condition were used. Then quality characteristics of fruits (weight loss, pH, TA, TSS, vitamin C, anthocyanin, calcium, pectin, catalase, peroxidase and polygalacturonase activity, decay and sensory analyses) were evaluated. Treated fruits with thymol had lower weight loss, pH, TSS, POD, PG and decay and higher TA, vitamin C, anthocyanin, calcium, pectin, CAT and fruit quality compared to controls. As a general result, thymol, as a non-chemical compound, in higher concentrations 500 and 1000 $\mu l l^{-1}$ caused lower decay incidence and longer storability and improved fruit quality compared to other treatments. Among all the treatment methods, paper disk method had higher effectuality compared to spray method.

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Introduction

Strawberries (*Fragaria ananassa*) are highly perishable fruits and characterized by a short storage life (Han *et al.,* 2005). Several naturally occurring essential oils have been identified to be effective in inhibiting microbial growth (Nychas, 1995) and have started to become an effective alternative to synthetic fungicides (Tripathi and Dubey, 2004). Postharvest application of fungicides is limited to adverse effects due to pesticide residues on food, along with pathogen resistance to many currently used pesticides and wetting the fruits and by stringent federal and state regulations concerning use of available fungicides (Ecket and Ogawa, 1988; Tripathi and Dubey, 2004). Thymol is a natural monoterpene phenol derivative of cymene, found in oil of thyme, and extracted from *T. vulgaris* and various other kinds of plants as a white crystalline substance and strong antiseptic properties (Moghtader, 2012). Thymol has microbial activity because of its phenolic structure. Thymol is listed by the food and drug administration (FDA) as foods for human consumption. It is considered generally recognized as safe GRAS (EPA, 1993). Moghtader (2012) reported that the high percentage antifungal activities of *Thymus* oil are related with thymol as the main compound. Treatment of strawberry with thymol, eugenol, or menthol significantly delayed deterioration of the fruit (Wang *et al.,* 2007) and improved fruit quality and safety in table grapes and sweet cherries (Serrano *et al.,* 2005; Valverde *et al.,* 2005). Wang *et al.* (2008) investigated the effects of carvacrol, anethole, cinnamaldehyde, cinnamic acid, perillaldehyde, linalool and ρ-cymene on increasing antioxidant activity and reducing decay of blueberries. Liu *et al.* (2002) also found that thymol was effective for controlling brown rot symptoms on apricot and can greatly reduce postharvest decay of plums without causing any phytotoxicity. In this study, the inhibitory effect of thymol against strawberry fruit decay was investigated which could improve fruit quality and shelf life.

Material and methods

Plant material

The physiologically mature strawberry fruits of 'Gaviota' cultivar were harvested in the morning randomly from a commercial greenhouse located in Hashtgerd, Karaj, Iran. Uniform and undamaged fruits were isolated from the bruised and damaged one and selected for further studies. Samples were washed with sodium hypocholorite solution to keep safe from microbial contamination.

Thymol treatment

Treatments with thymol were performed by dissolving the requisite amounts of thymol (0, 125, 250, 500 and 1000 µl/l) in ethanol. Two different methods were applied (spray thymol solution on fruits and paper disk method). Thymol sprayed on fruits and spotted onto filter paper at the final concentration of control, alcohol treated samples, 125, 250, 500 and 1000 $\mu l l^{-1}$ then air dried. Each treatment was replicated three times with 150 g fruits per replicate. All packages were stored at 4 °C and 85 % RH in darkness. Different quality attributes were observed during 12 days of storage. Measurements were made at room temperature every 3 days up to the end of experiment.

Weight loss percent

Weight loss was measured after 0, 3, 6, 9 and 12 days. Weight of individual fruits was recorded at the beginning of harvest as reference weight and different sampling times and calculated by using following formula (Ali *et al.,* 2011):

Weight loss (%) = initial weight – recorded weight / initial weight × 100.

pH

pH was determined using a pH meter Metrohm Lab 827, which had been previously standardized to pH 7 (Ali *et al.*, 2011).

Titratable Acidity (TA)

Titrable acidity was measured using titration method. To do that, 5 mL fruit juice was added to 25 mL distilled water plus two drops of phenolphthalein and titrated with 0.1N NaOH up to pH 8.1. The results were converted to percent citric acid and expressed in

terms of fresh weight (AOAC, 1990).

Total soluble solids (TSS)

TSS was determined using ATAGO-ATC-20E (Japan) refractometer at and expressed as ºBrix.

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 × dye equiv. × dilution ×100/ Wt. of sample) (Saini *et al.,* 2006).

Anthocyanin assay

Total anthocyanin content of strawberry extract was determined using the pH differential method. 5 grams of fresh fruits were extracted with 25 mL of 80% acetone containing 0.2% formic acid. Absorbance was measured in a spectrophotometer at 510 and 700 nm, respectively, in different buffers at pH 1.0 and 4.5, using A = [(A510-A700) pH1.0 (A510-A700) pH 4.5] with a molar extinction coefficient for cyanidin-3-glucoside of 29600. Results were expressed as milligrams of cyanidin-3-glucoside (C3G) equivalents per 100 g of fresh weight (Cheng and Breen, 1991).

Calcium content

Calcium was precipitated as calcium oxalate. The precipitate was dissolved in hot dilute sulfuric acid and titrated with standard potassium permanganate. 1ml 0.1N $KMnO_4$=0.002 gm Calcium (Ruck, 1969).

Pectin content

Pectin was precipitated as calcium pectate from an acid solution by the addition of calcium chloride. The calcium pectate precipitate was washed with water until chloride-free, then dried and weighed. Ca pectate (%) = wt. of Ca pectate× 100 / wt. of sample (Ruck, 1969).

Peroxidase activity assay

POD activity was assayed spectrophotometrically with guaiacol by measuring an increase in absorbance at 470 nm (ε= 26.6 $mM^{-1}cm^{-1}$) according to Maehly and Chance (1954). The mixture of 0.5 cm3 of the enzyme extract, 0.5 cm3 of 50 mM acetate buffer (pH 5.6), 0.5 cm3 of 20 Mm guaiacol and 0.5 cm3 of 60 mM H2O2 was used. The enzyme activity was expressed in units (mmol tetraguaiacol min^{-1}) per g fresh weight.

Catalase activity assay

CAT activity was determined at 25 ° C according to Aebi (Aebi, 1984). The reaction mixture contained 40 mM phosphate buffer pH 7.0 and 0.1 ml pure enzyme in a total volume of 3ml. CAT activity was estimated by decreased in absorbance of H_2O_2 at 240nm.

Assay of polygalacturonase activity

PG activity was determined by measuring reducing groups released from sodium polypectate, using D-galacturonic acid as the standard. The assay medium reagents were 0.2 M acetate buffer, pH 4.5 to the amount 0.2 ml, and 1% polygalacturonic acid in 0.05 M acetate buffer solution pH 4.5 to the amount 0.3 ml. One ml of enzyme solution and distilled water was added. The reaction started by adding the enzyme, and it was then left for 30 min at 37° C, after which the reaction was stopped by adding 3, 5 – dinitrosalicylic acid (DNS). The solution was then boiled in water for 5 min, after which it was diluted and absorbance measured at a wavelength of 520 nm, using galacturonic acid (0–1mg/ml) as the standard solution (Miller, 1959). One unit of polygalacturonase activity (U/g) was defined as the amount of enzyme which released one mol of galacturonic acid per minute per gram of substrate.

Decay analysis

Percent of decay was scored on a 1-5 scale, where: 1= intact fruit, 2= more than 5 % Decay, 3= between 5-20 % decay, 4= between 20-50 % decay, 5= more than 50% decay (Ayala-Zavala *et al.*, 2005).

Sensory evaluation

Sensory analyses to compare the quality of treated and control fruits were carried out by a 10 trained adults aged 25-40 years. It was about aroma, taste, firmness, appearance and texture. Panelists scored

fruits between 1-10. 10 being the best total quality and 1 being the worst (Hernandez-Munoz *et al.,* 2008). Samples were scored for overall quality by using an interval hedonic scale. 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). The statistical analysis was performed using Microsoft Excel (2007) and SPSS software version 21 and means were compared using Duncan's Multiple Range Test (DMRT).

Results and discussion

Weight loss

In storage period, weight loss of fruits increased. Results showed that a significant decrease in weight loss percent was experienced in samples which were treated with the highest concentration of thymol (1000 µll^{-1}), compared to other concentrations (Fig. 1 and 2). No significant differences in weight loss were observed among 125, 250 and 500 µll^{-1} concentrations. Thymol treatment decreased weight loss of strawberries during storage for 12 days compared with controls. Between two methods of treatments fruits that treated with spray method (Fig. 2) had more weight loss than paper disk method (Fig. 1). In strawberry fruits the thin skin makes them susceptible to rapid water loss, resulting in shriveling and deterioration (Hernandez-Munoz *et al.,* 2008). Due to the thickness of polyethylene package a solid barrier will develop against water steam's exit. It seems that the weight loss of sample during storage is due to water pressure differences between sample and surrounding air, which causes the sample's tissue to lose weight. It appears that essential oil could act as a barrier against water steam's exit.

pH

The pH value of strawberry fruit increased slightly, corresponding to a decrease in TA during storage. Little difference in pH value was observed among 250, 500 and 1000 µll^{-1}. Control fruits had the most pH value and 125 µll^{-1} concentration of thymol had the least pH value. No significant differences were observed between methods of treatments application (Fig. 3). It is expected that a relationship exists between the level of acidity and pH. Results obtained were in consistent with those obtained by Ali *et al.* (2011) who stated pH of strawberry fruits was almost stable during storage in all treatments. In present study, treated fruits had lower pH compared with controls.

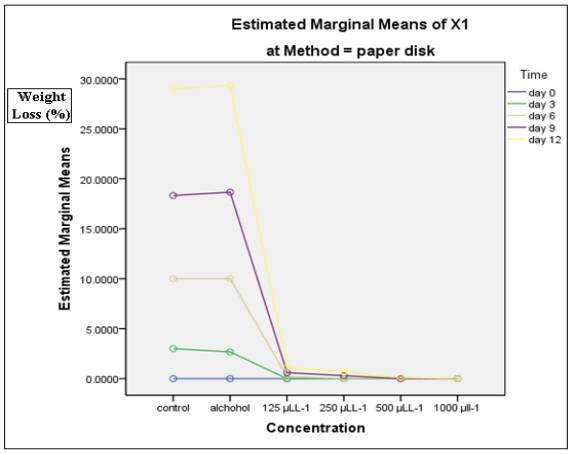

Fig. 1. The effect of thymol treatment on weight loss of strawberry fruit in paper disk method.

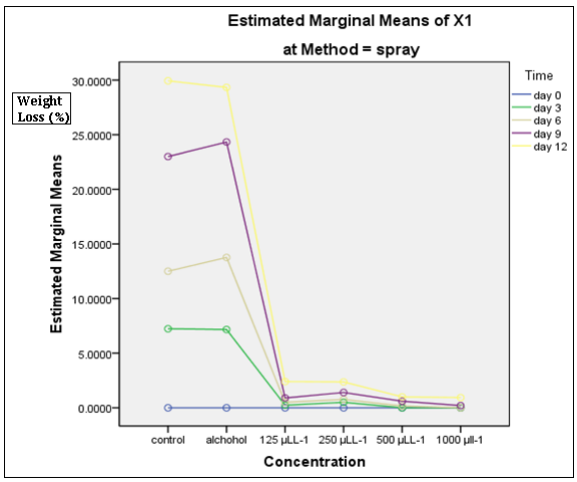

Fig. 2. The effect of thymol treatment on weight loss of strawberry fruit in spray method.

Titratable acidity

TA decreased gradually during storage. TA of 250 µll^{-1} concentration of thymol was the highest among all of concentrations and controls had the lowest TA amount. Fruits that treated with paper disk method

had more TA content compared with spray method (Fig. 4 and 5). Acidity declines as fruit mature. The differences found in pH and TA during storage between treated and untreated fruits could be related to the greater loss of water by untreated fruits. TA has been expressed as milligrams of citric acid per gram of fresh weight. The results are in agreement with Ali *et al.* (2011) and Nunes *et al.* (1995).

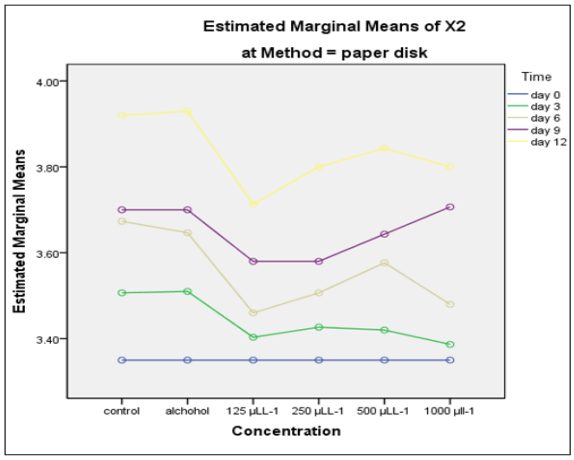

Fig. 3. The effect of thymol treatment on pH of strawberry fruit.

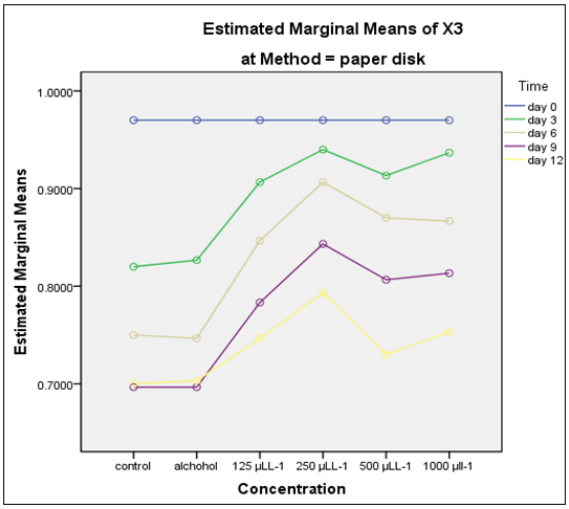

Fig. 4. The effect of thymol treatment on titratable acidity of strawberry fruit in paper disk method.

Total soluble solids

While time passed, TSS rate increased. Results showed that among all concentrations of thymol 250µll^{-1} had the lowest TSS amount and controls had the highest amount of TSS (Fig. 6) which are in agreement with Raafat *et al.* (2012) who obtained thyme oil significantly decrease the TSS values of strawberry fruit. Depletion of TSS could be explained by a high metabolism of fruit and senescence processes. Lower rates of respiration of strawberries treated with thymol might have contributed to conserve higher levels of carbohydrates in tissue (Ayala-Zavala *et al.,* 2005) Hernandez-Munoz *et al.* (2008) stated that loss of water suffered by strawberries during storage increased TSS level of fruits and the greater changes in TSS occurred in those fruits which suffered the greatest water loss which is in accordance with our results.

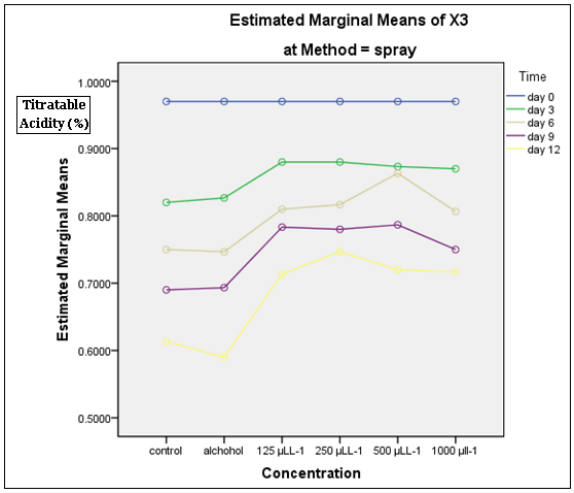

Fig. 5. The effect of thymol treatment on titratable acidity of strawberry fruit in spray method.

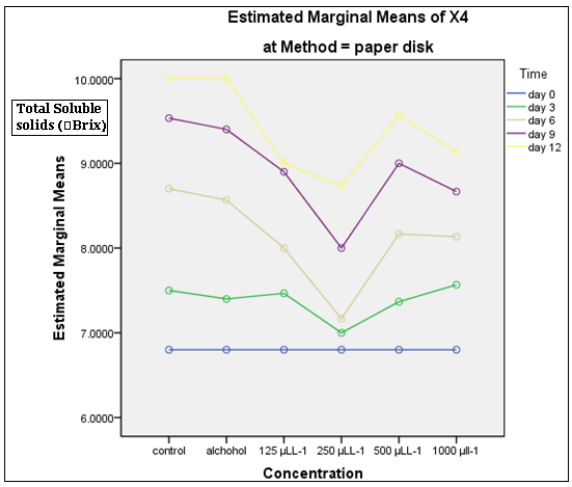

Fig. 6. The effect of thymol treatment on total soluble solids of strawberry fruit.

Vitamin C

Vitamin C content of fruits decreased significantly along the storage period. The samples which were treated with 1000 µll^{-1} thymol had the highest vitamin C and samples with125 µll^{-1} thymol had the lowest vitamin C compared to others. Control fruits had the

lowest amount of vitamin C. It seems that the increase in thymol concentration can enhance fruit vitamin C. Fruits that treated with paper disk method had more vitamin C content compared with spray method (Fig. 7 and 8). This finding was inconsistent with results obtained by Raafat *et al.* (2012) who stated thyme oil significantly decrease vitamin C content of strawberry fruits. Ascorbic acid will be oxidized easily by the ascorbate oxidase enzyme and changes into inactive form (Lee & Kader, 2000).Vitamin C content of treated fruits with eugenol and thymol decreased along the storage (Valero *et al.*, 2006). These finding were in agreement with results obtained in our experiment.

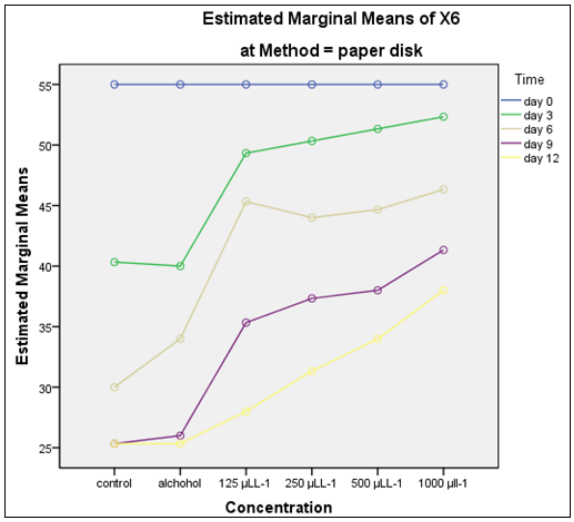

Fig. 7. The effect of thymol treatment on vitamin C of strawberry fruit in paper disk method.

Anthocyanin

Anthocyanin content decreased during storage period. The levels of anthocyanin were higher in thymol-treated fruits than in control samples, whereas the highest concentration (1000 µll^{-1}) of thymol treated fruits had the most amount of anthocyanin. As shown in Fig. 9 and 10 there were significant differences between two methods of thymol application. Fruits treated with paper disk method preserved more anthocyanin compared with spray method. Anthocyanins are a group of phenolic compounds responsible for the red-blue color of many fruits. In accordance with our results, Wang *et al.* (2008) reported all essential oil treatments enhanced the levels of blueberries anthocyanin compared to controls.

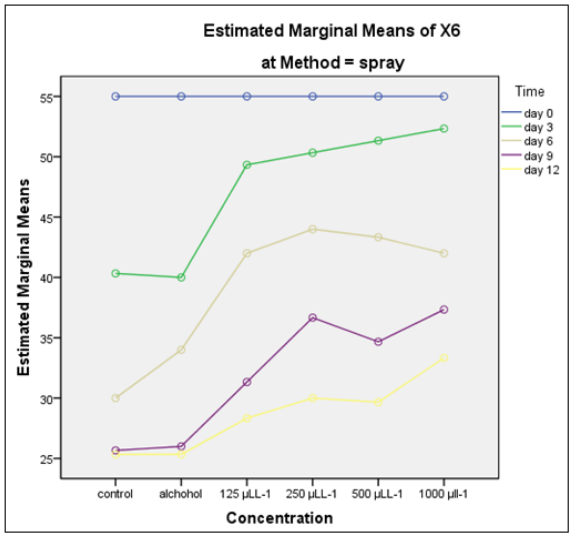

Fig. 8. The effect of thymol treatment on vitamin C of strawberry fruit in spray method.

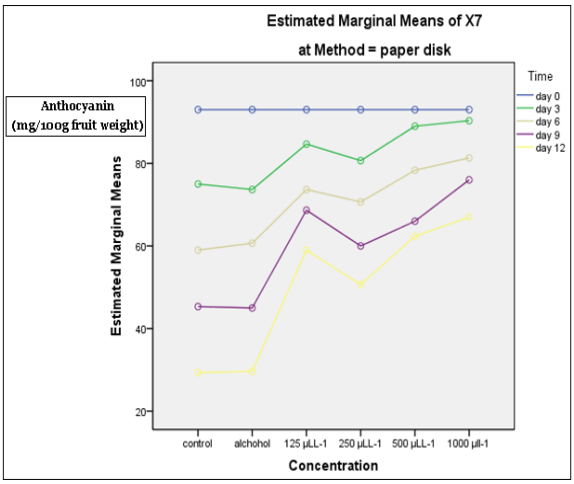

Fig. 9. The effect of thymol treatment on weight loanthocyanin of strawberry fruit in paper disk method.

Calcium and pectin contents

Calcium and pectin contents decreased gradually during storage period. Thymol-treated fruits had more calcium and pectin content compared to controls. Fruits treated with paper disk method had higher amount of calcium and pectin compared with spray method (Fig. 11-14). The effect of thymol treatment on fruit calcium and pectin significant varied with the concentrations applied. As shown in Figures fruits treated with 1000 µll^{-1} had the most amounts of calcium and pectin and the lowest amounts of calcium and pectin were in control fruits. Pectins are likely to be the key substances involved in

the mechanical strength of the primary cell wall which are important to the physical structure of the plant (Sirisomboon *et al.,* 2000). Their degradation during ripening seems to be responsible for tissue softening and leads to the disassembly of cellulose-hemicellulose network and accelerates the fruit softening rate (Duan *et al.*, 2008). According to Koh and Melton (2004) softening of strawberry fruit during storage is mainly due to loss of cell wall material. Softening of strawberry is mainly due to the presence of polygalacturonase which solubilizes and degrades the cell wall polyuronides (Huber, 1984). Hernandez-Munoz *et al.* (2008) reported that calcium can delay the fungal decay of several commodities, including strawberries.

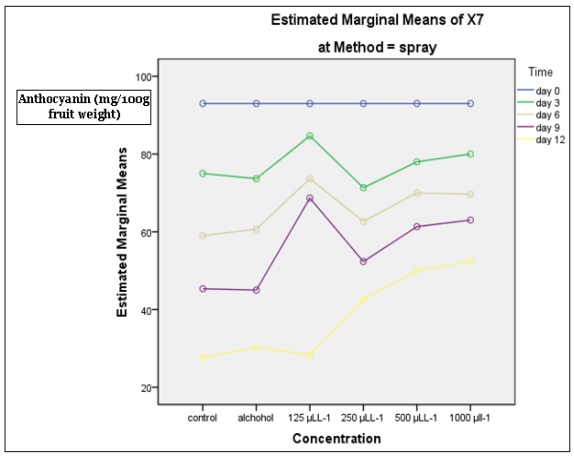

Fig. 10. The effect of thymol treatment on anthocyanin of strawberry fruit in spray method.

Sensory evaluation

Fig. 15 and 16 shows the effect of thymol on overall quality of strawberries. Overall quality decreased continuously during storage at higher rate in untreated fruits compared with those treated with thymol. Treatment with 500 and 1000 µll^{-1} concentrations of thymol had the highest effect on fruit quality among all of concentrations. Fruits that treated with paper disk method had better fruit quality compared with spray method. Our results were in accordance with Liu *et al.* (2002) who found that thymol in relatively low concentrations such as 2 or 4 mg/l can greatly reduce postharvest decay without causing any phytotoxicity. The greater visual acceptance by consumers for treated fruits correlates with the lower amount of dehydration and darkening experienced by them during storage period. In this regard, thymol treatment represents an alternative means to enrich the quality and nutritional value of strawberry fruit.

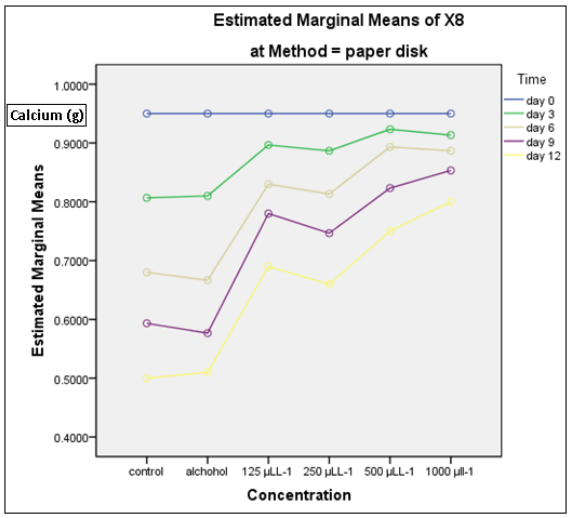

Fig. 11. The effect of thymol treatment on calcium content of strawberry fruit in paper disk method.

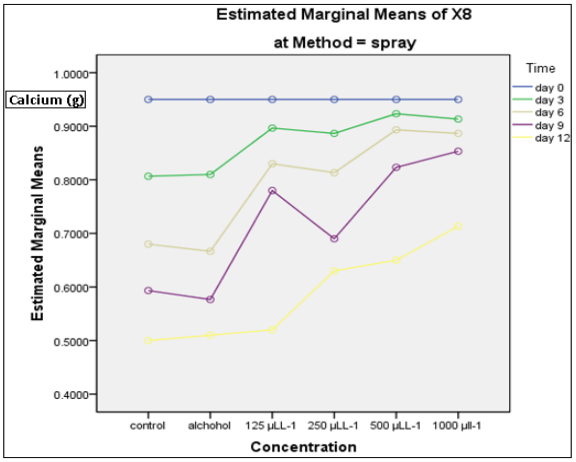

Fig. 12. The effect of thymol treatment on calcium content of strawberry fruit in spray method.

Catalase activity

Result showed that the catalase activity decreased at the end of the storage period. The controls had the lowest catalase activity and the samples which were subjected to thymol with 500 and 1000 µll^{-1} concentrations had the highest catalase activity (Fig. 17). Catalase eliminates H_2O_2 by breaking it down directly to form water and oxygen. Fruits subjected to thymol with 500 and 1000 µll^{-1} prevented from accumulation of H_2O_2. Puntarol *et al.* (1988) showed that the catalases are responsible for H_2O_2

elimination and that an over activity exhibition of these enzymes is able to induce hydrogen peroxide detoxification and is considered as a strategy for improving tolerance to the stress for plant cells.

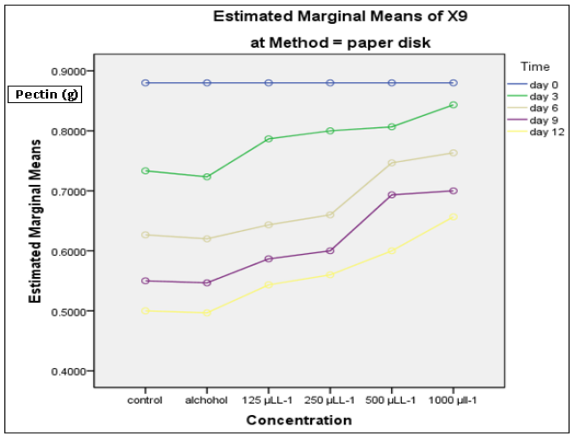

Fig. 13. The effect of thymol treatment on pectin of strawberry fruit in paper disk method.

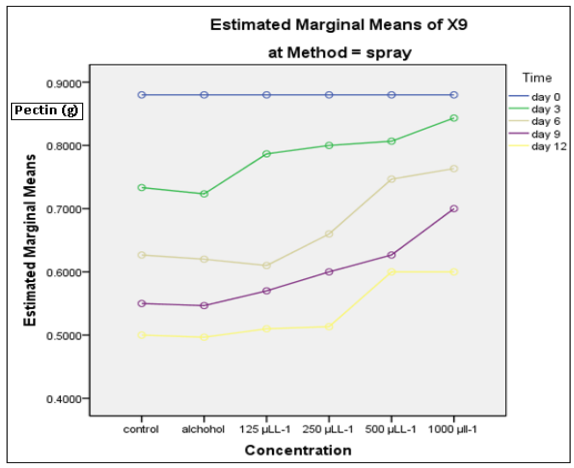

Fig. 14. The effect of thymol treatment on pectin of strawberry fruit in spray method.

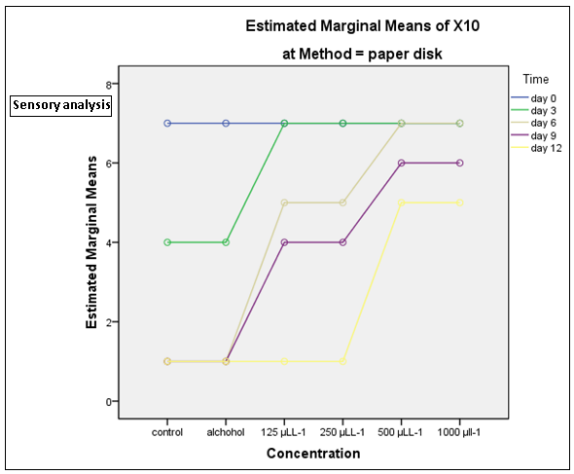

Fig. 15. The effect of thymol treatment on sensory analysis of strawberry fruit in paper disk method.

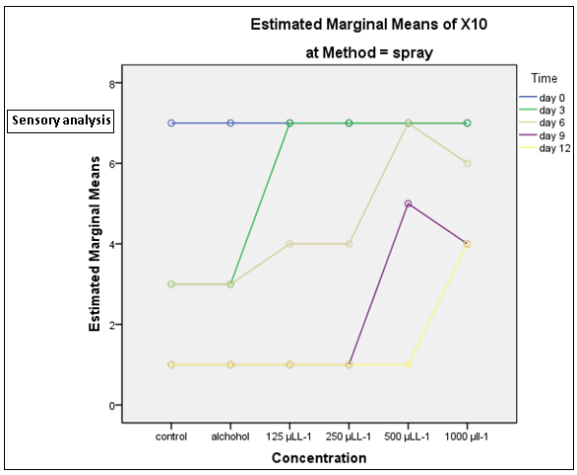

Fig. 16. The effect of thymol treatment on sensory analysis of strawberry fruit in spray method.

Peroxidase activity

Results showed that there was no significant increase in peroxidase activity until 3 days of storage. After that the peroxidase activity increased along the storage. The samples with concentration 1000 $\mu l l^{-1}$ showed a significant decreased in POD rate (Fig. 18). Peroxidase (POD) activity plays an important role in the oxidative degradation of phenolic compounds, which can lead to the production of brown polymers (Tomás-Barberán and Espín, 2001). It seems that thymol prevented the POD activity in treated samples.

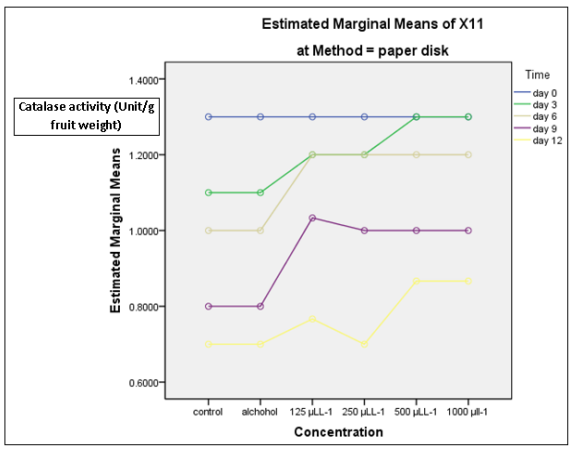

Fig. 17. The effect of thymol treatment on catalase activity of strawberry fruit.

Polygalacturonase activity

In accordance with Venkatesan and Tamilmani (2010), PG content of fruits increased significantly along the storage period. The controls had the highest PG activity and the samples which were subjected to thymol had less PG activity compared to controls (Fig.

19 and 20). The samples which were treated with 1000 µll^{-1} thymol had the lowest PG activity. PG is an enzyme involved in pectin metabolism during fruit ripening and is associated with cell wall breakdown and loss of tissue integrity. It can be concluded that thymol protected fruits from textural softening during ripening and decreased the PG activity. Therefore, tightly bound protopectin didn't degrade into soluble pectin, which is found loosely bound to the cell walls and treated fruits protected from textural softening during storage (Venkatesan and Tamilmani, 2010).

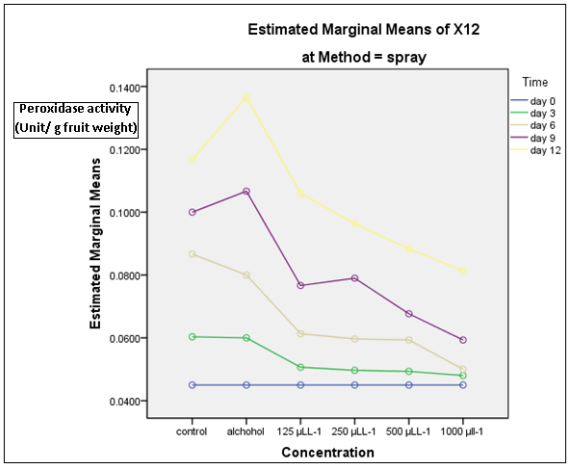

Fig. 18. The effect of thymol treatment on peroxidase activity of strawberry fruit.

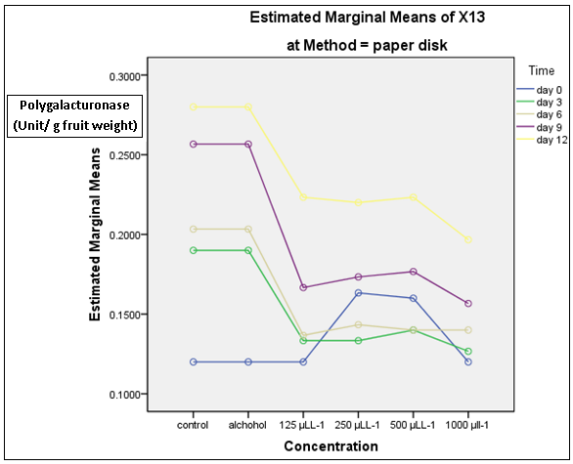

Fig. 19. The effect of thymol treatment on polygalacturonase activity of strawberry fruit in paper disk method.

Fruit decay

The effect of thymol treatment on fruit decay significant varied with the concentrations applied and storage time. Treatment with 500 and 1000 µll^{-1} thymol significantly inhibited fruit decay throughout the storage period, whereas control samples had the least effect. Paper disk method inhibited fruit decay more than spray method (Fig. 21 and 22). Antifungal activity of vapor phase of thymol (paper disk method) is result of indirect effect of it on mycelium and lipophilic properties that provide the opportunity to be absorbed by the mycelium. Our results are in accordance with Soylu *et al.* (2006) that stated antifungal activity of rosemary essential oil in vapor phase is more than contact method. Strawberry fruit has a very short shelf life, mostly due to their relatively high water content, high metabolic activity, and the susceptibility to microbial molds and rots. Our results were in agreement with Wang *et al.* (2008).

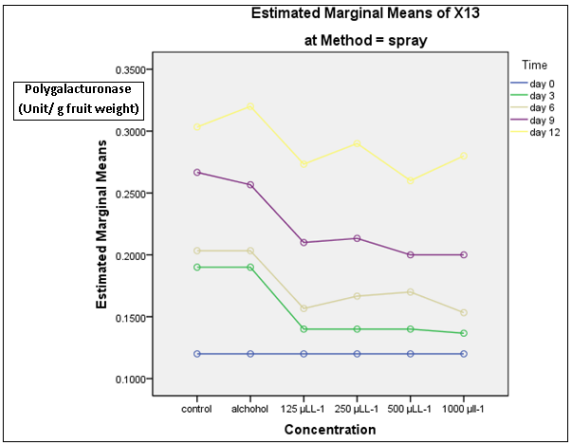

Fig. 20. The effect of thymol treatment on polygalacturonase activity of strawberry fruit in spray method.

The antifungal effect of thyme oil by paper disk method against *Botrytis cinerea* in strawberry fruit was reported by Raafat *et al.* (2012). Abd-Alla *et al.* (2011) suggested that thyme oil vapor was the most effective treatment which reduced gray mold of strawberry fruit. The microbial mode of action of thymol has been postulated as disruption of cellular membrane functions and interference with active sites of enzymes and cellular metabolism (Farag *et al.,* 1989; Marino *et al.*, 2001) and may change the permeability of membranes of the microbes for cations and alter the ion gradients that lead to impairment of vital processes in cells and eventually cell death (Ultee *et al.,* 1999). Thymol with phenolic

group as its major component have shown enhanced the shelf life of strawberry fruits during storage time by protecting them from grey mold. The fungicidal or fungistatic activity of the essential oil of *Thymus vulgaris* can be attributed to thymol, especially the hydroxyl group of this compound (Lira Mota *et al.* 2012).

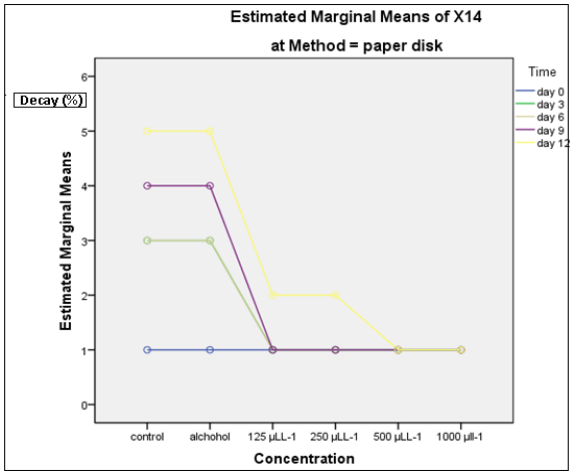

Fig. 21. The effect of thymol treatment on decay percentage of strawberry fruit in paper disk method.

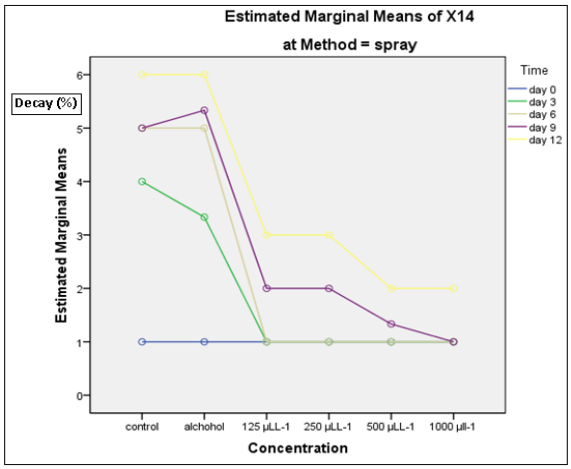

Fig. 22. The effect of thymol treatment on decay percentage of strawberry fruit in spray method.

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