

Improving Strawberry Fruit Storability by Edible Coating as a Carrier of Thymol or Calcium Chloride

¹Amal, S.H. Atress, ²M.M. El-Mogy, ³H.E. Aboul-Anean and ⁴B.W. Alsanis

¹Department of Vegetable Handling, Hort. Res. Institute, Agric. Res. Center, Giza, Egypt

²Department of Vegetable Crops, Faculty of Agriculture, Cairo University, Egypt

³Department of Manufacturing Eng. Packaging Food Tech. Res. Institute, Agric. Res. Center, Giza, Egypt

⁴Microbial Horticulture Laboratories, Department of Horticulture, SLU, Sweden

Abstract: The aim of this study was to improve strawberry fruit storability by testing the effect of edible coating with soy or wheat gluten protein as a carrier of thymol, which mainly presents in the essential oil of thyme plants and calcium chloride on quality of strawberry fruits. Thymol and calcium chloride were applied during preparing the edible coating film. Coating fruits with thymol carried by soy protein or white gluten and CaCl₂ carried by soy protein showed the lowest weight loss percentage. Treating fruits with thymol carried by soy protein or white gluten did not exhibit any change in fruit appearance until 9 days of storage. All treatments maintained ascorbic acid content, firmness, TSS, total sugar and reduced the total colony, molds and yeasts compared to control. Fruits coated with thymol carried by soy protein or white gluten was the most effective treatments. In addition, coating fruits with thymol carried by soy protein or white gluten recorded the lowest values of anthocyanin, higher chroma and higher hue angle compared to control fruits.

Key words: Calcium chloride • Edible coating • Microbial counts • Quality • Soy protein • Strawberry • Thymol • Wheat gluten

INTRODUCTION

Strawberry is a non-climacteric fruit with a very short postharvest shelf-life. Loss of quality in this fruit is mostly due to its relatively high metabolic activity and sensitivity to fungal decay, mainly grey mold (*Botrytis cinerea*). Strawberries are also susceptible to water loss, bruising and mechanical injury due to their soft texture and lack of a protective rind. To reduce spoilage, metabolism, deterioration and to extend the shelf life, strawberry fruit should be kept at 0°C after harvest [1].

The postharvest life of strawberries can be extending by several techniques combined with refrigeration. Edible coatings have long been known to protect perishable food products from deterioration [2]. The purpose is to extend the shelf life of produce and to provide a barrier against hazards. It may retard moisture migration and the loss of volatile compounds, reduce the respiration rate and delay changes in textural properties [3]. Also, it displays an excellent barrier to fats and oils and has a high selective gas permeability ratio CO₂/O₂ as compared to conventional synthetic films [4].

Essential oils have shown a substantial potential to controlling the postharvest decay of vegetables and might act as alternatives to synthetic fungicides. Essential oils produced by different genera are, in many cases, biologically active, endowed with antimicrobial, allelopathic, antioxidant and bioregulatory properties [5]. The essential oils extracted from various scent plants have been shown to have significant antifungal properties [6].

Thymol, one of the most important essential oils, is highly active against a broad spectrum of microorganisms. Calcium plays an important role in forming cross-bridges which influence cell wall strength and is regarded as the last barrier before cell separation. Calcium maintains the cell wall structure in fruit by interacting with the pectic acid in the cell walls to form calcium pectate. Therefore, a greater extent of Ca²⁺ bonding in pectic polymers will reduce the rate of pectic solubilization and accordingly the loss of pectin stabilization by Ca⁺² may contribute to fruit softening [7]. Postharvest application of calcium prevents postharvest disorders, retards fruit ripening and decreases postharvest decay [8]. Moreover, strawberry fruit treated with calcium had increased

calcium content in the cell wall of the fruit tissue and maintained fruit firmness and soluble solid contents, without affecting their sensory quality [9, 10].

The aim of this work was to evaluate the effectiveness of soy and wheat gluten proteins edible coating film with either thymol or calcium chloride as carries to improve strawberry fruit storability under cold storage. Also, it is aimed to minimizing decay and microbial growth during the storage period.

MATERIALS AND METHODS

Strawberries (*Fragaria x ananassa* cv. Festival), were grown in a local farm on loamy soil (Elkalubia Governorate, Egypt) and received the normal agriculture practice during the two successive seasons (2008 and 2009). The substances used in this experiment were soy protein (acquired from the Food Technology Research Institute, Agriculture Research Center, Giza, Egypt), wheat gluten (from Dough and Bread Research Department, food Technology Research Institute, Agriculture Research Center, Giza, Egypt), calcium chloride (Coo 441/1, ADWIC Comp., Egypt.), ethanol (Eoo5811, 95%, ADWIC, Comp., Egypt), sodium hydroxide (un/1823 Chemicals Comp., UK), glycerin (P05650, El-Gomhouria Comp., Egypt.), ammonium hydroxide (010276, Jenapharm, Germany), citric acid (8010295, ADWIC, Comp., Egypt), butyle hydroxy tolween (Acros Organics, Belgium) and calcium hypo chlorides (El-Gomhoria Comp., Egypt).

Soy Protein Coating Formation: Film-formation solution was prepared by dissolving 5 g of soy protein with 2.5 g of glycerin in 100 ml distilled water. Solution pH was adjusted to pH 10 using sodium hydroxide (2N). Then, the solution was heated to 75°C for 15 min according to Kim *et al.* [11]. In this trial, the described film formation solution mentioned above was modified by adding 1 ml of thyme essential oil or CaCl₂ (1%, w/v), respectively.

Wheat Gluten Film Formation: The wheat gluten film was prepared by dissolving 9 g of gluten and 1.5 g of glycerol in 32.5 ml ethanol 98% + 67.5 ml of distilled water using a heating magnetic stirrer at 70°C and centrifuged at 300 rpm for 15 min. in room temperature. The pH solution was adjusted to pH 10 using ammonium hydroxide [12]. In this trial, the described film formation solution mentioned above was modified by adding 1 ml of thyme essential oil or CaCl₂ (1%, w/v), respectively.

Uniform strawberry fruits, in size and free from physical damage or fungal infection, were harvested at ¾ surface color stage, packed in field box and transported to the lab. Fruits were stored overnight at 0°C. In the next day, the strawberry fruits were washed with tap water and then immersed for 2 min. in disinfectant solution of calcium hypochlorite (0.25g L⁻¹). The fruits were divided into five treatments as follows:-

- T1 : Control: dipping fruits in distilled water,
- T2 : Soy protein plus thymol coating film,
- T3 : Wheat gluten plus thymol coating film,
- T4 : Soy protein plus CaCl₂ coating film and
- T5 : Wheat gluten plus CaCl₂ coating film.

Dipping period for each treatment was one minute. Fruits were dried after dipping and packaged in plastic trays with approximately 250 g of strawberries per tray. Each treatment contained 8 trays and each tray was considered as one replicate. Each treatment (8 trays) was packed in one cartoon box. After that, all boxes were stored at 0°C and 90-95% RH for 15 days. The following parameters were recorded:

Weight Loss: Strawberry fruits were weighed at zero time of the storage beginning and 3-day intervals during the storage period. Weight loss was determined as a percentage referring to index weight at zero time according to Hen *et al.* [13].

General Appearance: General appearance was obtained by submitting samples to 5-member panel experienced in judging sensory analysis of vegetables. Samples were identified with random numbers and arranged on individual plates. Each sample was rated using score system as follows: 9 = excellent, 7 = good, 5 = fair, 3 = poor and 1 = unsalable) as described by Kader *et al.* [14]. This scale describes fresh appearance, fresh calyx, change of color and decay. General appearance rating of 5 was considered as the limit for salability.

Firmness: Fruit firmness (by g cm⁻²) was determined by measuring the compression force (at 0.05 mm s⁻¹) of the samples using a texture analyzer according to Hen *et al.* [13].

Skin Color Measurement: Skin color was measured using a Minolta Chroma Meter, model CR-200. Calibration was done by a white plate before use. Color changes were quantified in the L* C* and h°. L* refers to the

lightness, ranging from 0 = black to 100 = white, chroma represents color saturation which varies from dull (low value) to vivid color (high value) and hue angle is defined as a color wheel, with red-purple at an angle of 0°, yellow at 90°, bluish-green at 180° and blue at 270° [15].

Titrateable Acidity, Ascorbic Acid and Anthocyanins:

Titrateable acidity (TA) was determined by using 10 g aliquots of strawberry fruits poured in 50 ml of distilled water and titrated with 0.1N NaOH to an end-point of pH 8.1. TA was expressed as percentage of citric acid and was calculated using the method reported by Hen *et al.* [13]. The ascorbic acid content was determined by using 2, 6-dichlorophenol indophenol titration method as described in A.O.A.C. [16]. Total anthocyanins were extracted by adding a solvent containing ethanolic HCl {95% ethanol, 1.5N HCl (85:15)}. The solvent was added at level (2:1) solvent to sample then the mixture was stored overnight at 4°C, then filtered on filter paper Watman No.1 and centrifuged at 1000 rpm for 15 minutes [17]. The supernatant intensity was measured by spectrophotometer (model Spectro UV-Vis 0216 USA).

Total Sugar and Total Soluble Solids: Total sugar was determined in fresh strawberry fruits by using Lane and Eynon method according to A.O.A.C. [16]. Total soluble solids was determined by the refractometric method at room temperature using an Abbe refractometer (Carl-Zeiss Jena) in juice pressed from a sample of homogenized strawberry slices [18].

Microbiological Analysis: Total microbial counted and moulds and yeasts were determined according to Marshal [19].

Preparation of Sample for Microbiological Analysis:

Under aseptic conditions, 50 g of each replicate were added to 450 ml of sterilized peptone water (1 g l⁻¹) in sterilized glass blender jar and blended for 5 min. Appropriate serial dilutions were done and then 10 ml of every samples were plated by standard microbiological pour plate technique. All the microbiological counts were carried out in duplicates.

Heterotrophic Bacterial Fena (Total Colony): Total colony count of bacteria was estimated using plate count agar medium according to the procedures, described inoculation and pour plating; 1ml of each dilution was pipetted into each of appropriately marked duplicate Petri dishes. Around 15-20 ml of plate count agar medium

{peptones (B.N. 025323, Bury, England) (5 g), glucose (G0058111, Adwic, Egypt) (1 g), yeast extract (B.N. 814372, Hampshire, England) (2.5 g), agar (B. N. 011391/843, Bury, England) (15 g) and distilled water (1 L). pH 7.0, sterilized by autoclavation for 15 minute at 121°C} were poured into each Petri dish, cooled to 45°C mixed thoroughly and allowed to solidify. The plates were incubated at 37°C for 48 hours. When the colonies were more than 30, the colonies were counted in both plate of a dilution and the average was calculated.

Moulds and Yeasts: The mould and yeast were determined using the methods for the microbiological examination of foods described by American Public Health Association [20] by using malt extract agar medium {malt extract (30 g), peptone (5 g), agar (15 g) and distilled water (1 L). pH 4 and sterilized by autoclaved for 15 minute at 121°C}, incubation at 20-25°C for 5 days if excessive growth develops, count colonies first after 3 days and then again after 5 days and reported as mould and yeast count per/g fresh weight.

Statistical Analysis: The obtained data were subjected to analysis of variance. The mean values were compared using LSD method at 5% level. The data were tabulated and statistically factorial analyses according to Snedecor and Cochran [21]. For microbial experiment, colonies were counted and the results expressed as cfu g⁻¹.

RESULTS AND DISCUSSION

Weight Loss Percentage: Weight loss percentage increased significantly with the prolongation of the storage period for all treatments (Fig. 1 a). Normally, the weight loss occurs during the fruit storage due to its respiratory process, the transference of humidity and some processes of oxidation [22]. However, the all treatments significantly reduced the weight loss of strawberries during the storage compared to the control. Comparing all treatments, thymol carried by soy or gluten and soy plus CaCl₂ treatments significantly reduced weight loss of strawberries. Edible coating are selective barriers to O₂ and CO₂, modifying internal atmospheres and slowing down the respiration rate of fruit, which in turn reduced weight loss [2]. The interaction between treatments and storage period was significant; however, soy plus thymol treatment was the most effective of all coatings showing a lowest weight loss after 15 days from storage. A similar effect was observed by Tanada-Palmu and Grosso [23] for strawberry fruits.

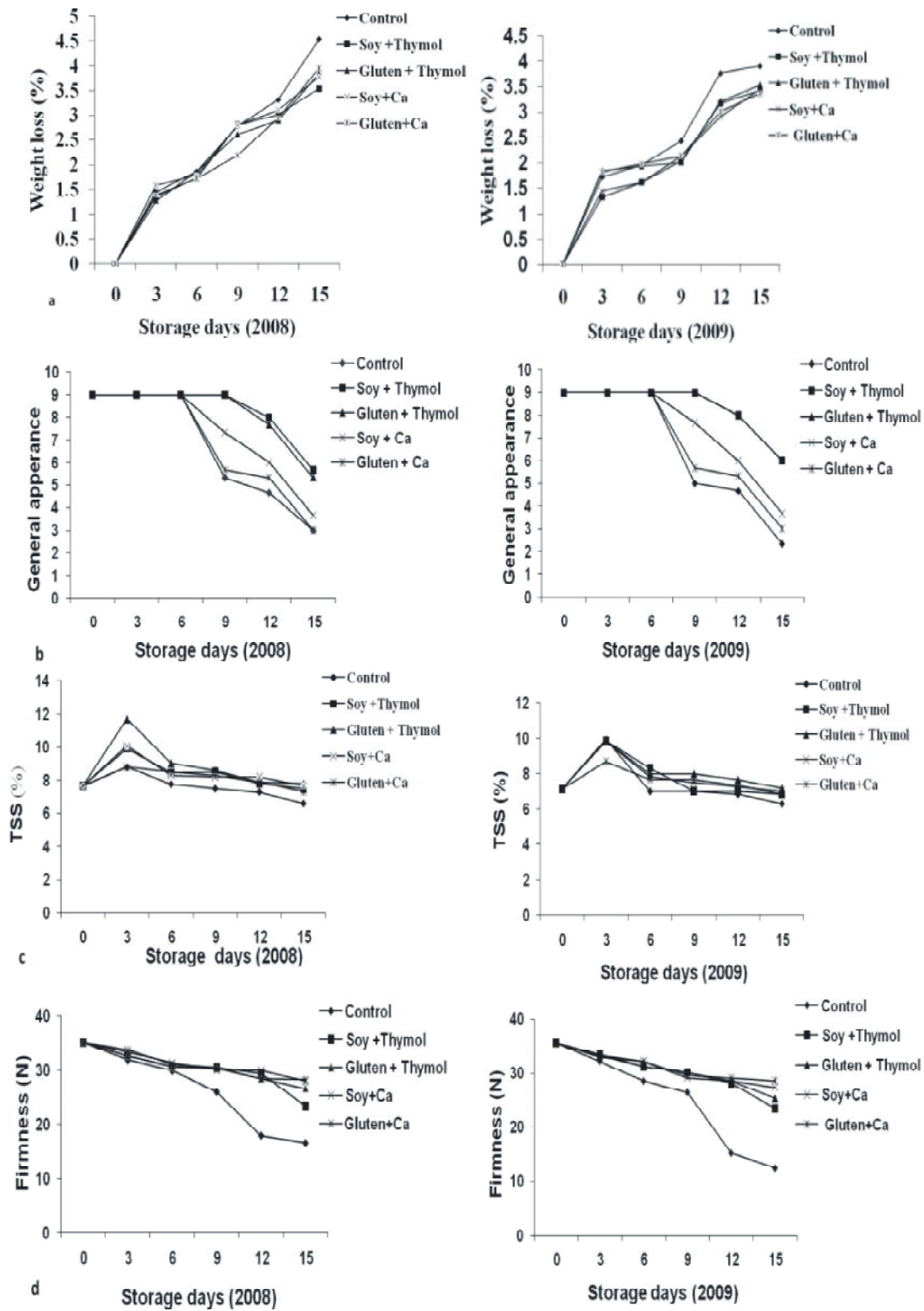


Fig. 1: Effect of edible coating on (a) weight loss [$LSD_{0.05} = 0.19$ (2008), 0.26 (2009)], (b) general appearance [$LSD_{0.05} = 0.36$ (2008), 0.37 (2009)], (c) TSS [$LSD_{0.05} = 0.23$ (2008), 0.25 (2009)] and (d) firmness [$LSD_{0.05} = 0.65$ (2008), 0.67 (2009)] on of strawberry fruits in 2008 and 2009 seasons.

General Appearance (GA): As expected, general appearance of strawberry fruits decreased during storage and the GA score dropped gradually from excellent to good, fair and poor, (9, 7, 5, 3) (Fig. 1 b). Both treatments coated with thymol carried by either soy protein or gluten film maintained the appearance until nine days of storage and displayed fruits with excellent to good appearance after 12 days of storage at 0°C. The general appearance of fruits coated with soy plus CaCl₂ decreased after six days of storage and dropped to poor level at the end of storage. On the other hand, strawberry fruits treated with gluten plus CaCl₂, as well as, untreated fruits (control) resulted in poor appearance after 15 days of storage at 0°C.

Total Soluble Solids (TSS): TSS percentage of strawberries increased during the first 3 days and then decreased by prolonged the storage period (Fig. 1 c). This reduction might be due to the respiratory process. These results are similar with those obtained by Shin *et al.* [24]. All coating treatments significantly reduced the loss of TSS percentage compared to control. Moreover, thymol oil carried by soy protein or gluten film was the most effective treatments in maintaining TSS during storage. Calcium chloride was less effective than thymol oil. Coating film on the surface of strawberry reduced respiration rate and vital process, thus reducing the loss of TSS during storage [23]. The interaction between treatments and storage period was not significant in both seasons.

Firmness: Fruit firmness (Fig. 1 d) decreased with extending the storage period in all treatments. Strawberries soften considerably due to ripening which mainly occurs because of degradation of the middle lamella of the cell wall of cortical parenchyma cells [25]. All tested treatments significantly maintained firmness compared with control. The highest values of firmness were achieved in fruits coated with soy or gluten plus thymol at the end of the storage period. These results are in agreement with Tanada-Palmu and Grosso [23], they stated that edible coating showed a good result with respect to the retention of fruit firmness probably because this coating slowed down metabolism and prolonged the storage life. The favorable effect of CaCl₂ treatment in reduction of firmness loss of strawberries during storage may be due to the stabilization of membrane systems and formation of Ca-pectates, which increase the rigidity of the middle lamella and cell wall to increase

resistance for polygalacturonase activity [26]. The interaction between treatments and storage period was significant in the two seasons. In this concern, strawberry fruits treated by soy plus Ca maintained the fruit firmness for 12 days at 0°C, however untreated control maintained the firmness for 6 days from storage, then firmness decrease was more rapidly until the storage end (15 days).

External Color: The color was measured recording lightness (L^* value), chroma (intensity of color) and hue angle (h°). Lightness of the fruit was affected by storage time (Fig. 2. a). There was a significant decrease in L^* value with increase storage for all treatments, showing darker fruit with storage. Strawberry fruits treated with thymol carried by soy or gluten resulted in lighter color (high L^* value), while fruits treated with gluten plus CaCl₂ and uncoated fruits (control) had darker color (low L^* value). Coated and uncoated samples showed a significant increase in hue angle and chroma during storage period until 9 and 3 days of storage respectively and then decreased, as the storage period was prolonged (Fig. 2, b and c). Uncoated fruits (control) and fruits coated with gluten plus CaCl₂ developed a redder (intensity of color as noted by significantly lower values) and less hue angle values comparing to other samples. On the other hand, treatments coated with thymol carried by soy or gluten and soy plus CaCl₂, gave fruits with higher chroma (vivid) and hue angle (less red). These results were agreements with those obtained by Colla *et al.* [27], they found that strawberry fruits treated with edible coating delayed fruits senescence in which the external and internal color was lighter than that of uncoated fruits. Thus, the senescence delay, evidenced by the decrease in color changes, demonstrates the effectiveness of this coating.

Titrateable Acidity: Titrateable acidity significantly decreased as a function of storage time for all studied treatments (Fig. 2 d). However, all treatments used retained more acidity compared to untreated fruits (control) during storage. These results were significant only during the first season. Titrateable acidity retention was reported by Tanada-Palmu and Grosso [23], using strawberry fruits coated with gluten film. Slowing down the strawberry respiration rate by means of an edible coating could explain the delay in the use of organic acid in the enzymatic reactions of respiration [2]. The interaction between treatments and storage period were significant in the two seasons.

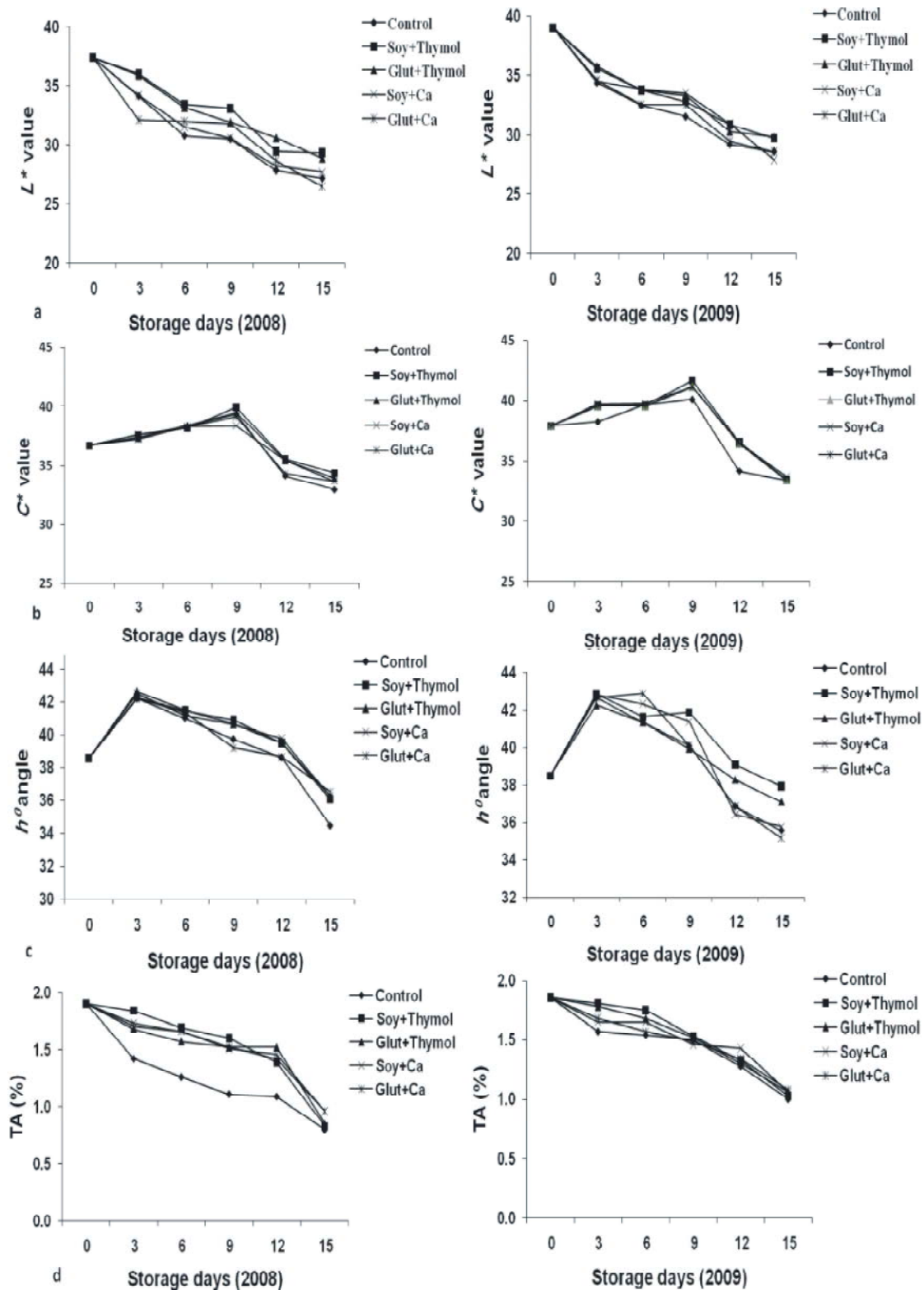


Fig. 2: Effect of edible coating on (a) L^* value [LSD_{0.05} = 0.52 (2008), 0.42 (2009)], (b) chroma [LSD_{0.05} = 0.25 (2008), 0.30 (2009)], (c) hue angle (h°) [LSD_{0.05} = 0.36 (2008), 0.31 (2009)] and (d) TA [LSD_{0.05} = 0.14 (2008), n.s. (2009)] of strawberry fruits in 2008 and 2009 seasons.

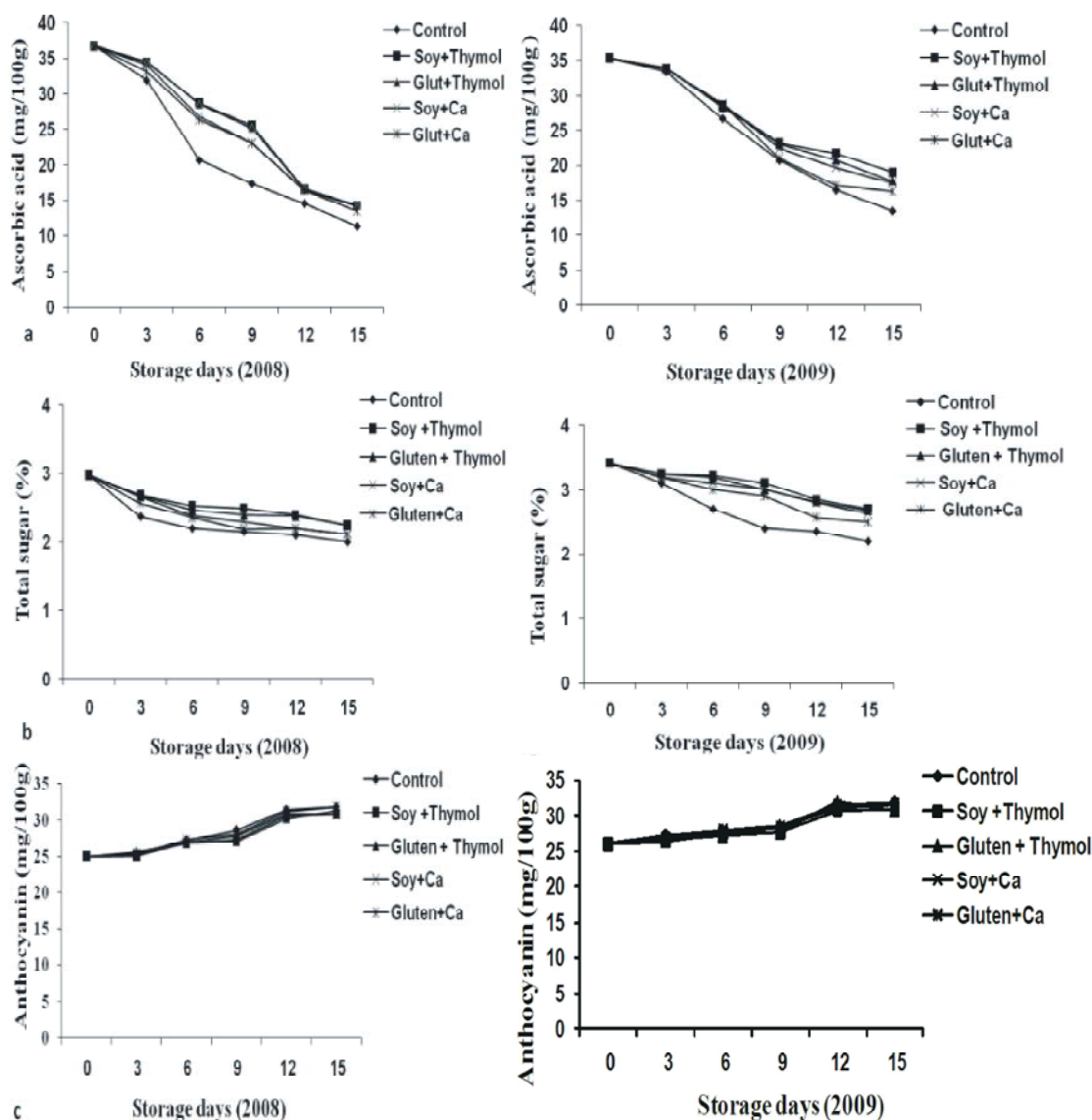


Fig. 3: Effect of edible coating on (a) ascorbic acid [LSD_{0.05} = 0.42 (2008), 0.40 (2009)], (b) total sugar [LSD_{0.05} = 0.08 (2008), 0.15 (2009)] and (c) anthocyanin [LSD_{0.05} = 0.54 (2008), 0.42 (2009)] of strawberry fruits in 2008 and 2009 seasons

Ascorbic Acid: Ascorbic acid content of strawberries was decreased by extending the storage period (Fig. 3 a). This reduction may be due to high respiration rate of strawberry fruits [28]. Various applied treatments had significantly maintained the ascorbic acid content in strawberries during storage compared to control, however, strawberries coated with thymol carried by soy or gluten significantly reduced the ascorbic acid loss of fruits followed by CaCl₂ carried by soy or gluten.

The lowering of vitamin C loss of strawberries treated with edible coatings attributed to slowing down the respiration rate of fruits, as affecting by coating [2],

which delays the deteriorative oxidation reaction of vitamin C. The favorable effects of CaCl₂ treatment in reduction of vitamin C loss of strawberries during storage may be due to that CaCl₂ prevents losses of antioxidant such as ascorbic acid in horticultural commodities [10]. The interaction between treatments and storage period was not significant during the two seasons.

Total Sugar: Total sugar content decreased with the prolongation of the storage period (Fig. 3 b). This reduction might be due to sugar loss through respiration [9]. Higher total sugar content of strawberries was

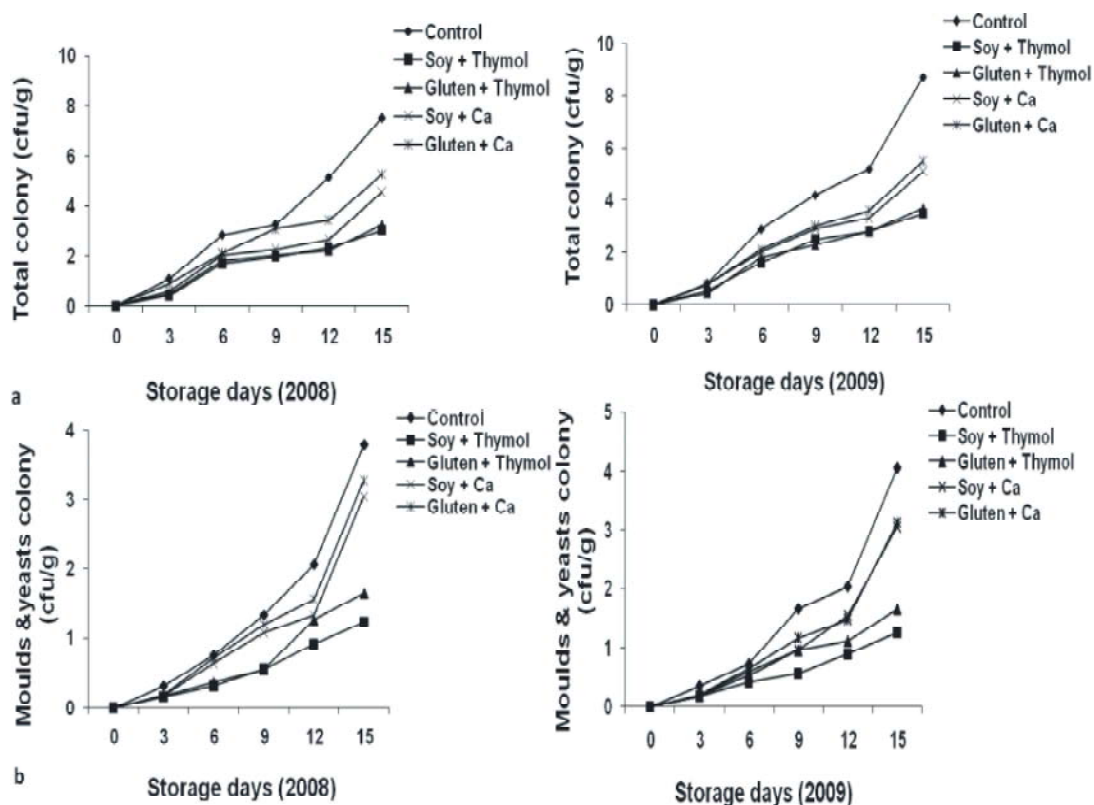


Fig. 4: Effect of edible coating on (a) total colony [LSD_{0.05} = 0.43 (2008), 0.41 (2009)] and (b) moulds and yeasts colony [LSD_{0.05} = 0.26 (2008), 0.42 (2009)] of strawberry fruits in 2008 and 2009 seasons

observed by coating treatments during the storage period compared to the control. Coated strawberries might reduce respiration rate; therefore, it can delay the use of total sugar in the enzymatic reactions of respiration [23]. The effect of calcium chloride on total sugar during storage might be due to calcium delayed the process as shown by increased contents in the cyclohexane-1,2 diamine tetra-acetate (CDTA) and Na₂CO₃ soluble fractions and lower levels in the water-soluble fractions.

Anthocyanin: Anthocyanin content of strawberry fruits were significantly increased during storage period (Fig. 3 c). These results were in agreement with those obtained by Hen *et al.* [13] and Holcroft and Kader [29], they found that fruits becomes redder and darker along the storage time, due to synthesis of anthocyanin, a pigment contributing to the red color in strawberry. However, coated treatment with thymol carried by soy or gluten and soy protein plus CaCl₂ gave the lowest values of anthocyanin content with insignificant differences between them, indicating that decrease in

color development and the fruits become less redness. On the contrary, gluten film plus CaCl₂ and uncoated treatment gave the highest values of anthocyanins, which developed more redness. The interaction between treatments and storage period were significant.

Heterotrophic Bacterial Fena and Moulds, Yeasts Colony:

Microorganism's growth was increased significantly as the storage period was prolonged particularly in control treatment (Fig. 4 a and b). All types of coatings had lower level of microbial load in comparison to control treatment. However, strawberries coated with thymol carried by soy protein or gluten film provided the lowest count in all types of microorganisms. Meanwhile, CaCl₂ treatment was less effective than thymol treatment in reducing this character. Also, the data revealed that strawberries coated with soy plus CaCl₂ treatment had less significant differences microorganism compare to fruits coated by gluten plus CaCl₂. These results are in agreement with those obtained by Zeringue and Bhatnagar [30], they found that thymol essential oil are more effective in inhibition of fungal growth and hence the mycelial weight.

Moreover, Rasooli and Abyaneh [31] clearly show that thymol essential oil related inhibition in mycelial growth was observed to be associated with significantly decreased levels of aflatoxin production. The reduction of microorganisms in strawberries treated with CaCl₂ may be due to calcium salts can lower intracellular pH or reduce water activity [32], which provides a protective antimicrobial barrier against food borne pathogens in product [33]. Also, microflora is usually restricted to fungal and lactic acid bacteria when pH was low [10]. Also, CaCl₂ treatment may have provided inhibitory effect on microbial growth [33].

REFERENCES

1. Hern'andez, P.M., E. Almenar, M.J. Ocio and R. Gavara, 2006. Effect of calcium dips and chitosan coatings on postharvest life of strawberries (*Fragaria x ananassa*). *Postharvest Biology and Technol.*, 39: 247-253.
2. Debeaufort, F.J.A. Quezada-Gallo and A. Voilley, 1998. Edible films and coatings: tomorrow's packagings: a review. *Crit. Rev. Food Sci.*, 38: 299-313.
3. Del-Valle, V., P. Hernandez-Munoz, A. Guarda and M.J. Galotto, 2005. Development of a cactus-mucilage edible coating (*Opuntia ficus indica*) and its application to extend strawberry (*Fragaria ananassa*) shelf-life. *Food Chemistry*, 91: 751-756.
4. Gontared, N.R., C. Thibault and S. Guilbert, 1996. Influence of relative humidity and film composition on oxygen and carbon dioxide permeability of edible films. *J. Agric. and Food Chemistry*, 44: 1064-1069.
5. Elakovich, S.D., 1988. Terpenoids as models for new agrochemicals. *ACS Symp. Ser.*, 380: 2580-2610.
6. Ahmet, C., K. Saban, K. Hamdullah and K. Ercan, 2005. Antifungal properties of essential oil and crude extracts of *Hypericum linarioides* Bosse, *Biochem. Syst. Ecol.*, 33: 245-256.
7. Fry, S.C., 2004. Primary cell wall metabolism: tracking the careers of wall polymers in living plant cells. *New Phytologist*, 161: 641-675.
8. Sadat, T., M. Shafiee, M. Babalar and K. Folta, 2008. 'Camarosa' strawberry postharvest quality is affected by hot water, salicylic acid and calcium dipping. *HortScience*, 43: 1149-1149.
9. Garcia, M.A., M.N. Martino and N.E. Zaritzky, 1998. Plasticized starch based coatings to improve strawberry (*Fragaria ananassa*) quality and stability. *J. Agric. Food Chem.*, 46: 3758-3767.
10. Luna-Guzman, S.J. and D.M. Barrett, 2000. Comparison of calcium chloride and calcium lactate effectiveness in maintaining shelf stability and quality of fresh-cut cantaloupe. *Postharvest Biol. and Technol.*, 19: 61-72.
11. Kim, K., C.L. Weller, M.A. Hann and A. Gennadios, 2002. Heat curing of soy protein films at atmospheric and sub atmospheric conditions. *J. Food Sci.*, 67: 708-713.
12. Patricia, S., T. Palmu, R. Carlos and F. Cross, 2005. Effect of edible wheat gluten-based films and coating on refrigerated strawberry (*Fragaria ananassa*) quality. *Postharvest Biol. and Technol.*, 36: 199-208.
13. Han, C., Y. Zhao, S.W. Leonard and M.G. Traber, 2004. Edible coatings to improve storability and enhance nutritional value of fresh and frozen strawberries (*Fragaria x ananassa*) and raspberries (*Rubus ideaus*). *Postharvest Biology and Technol.*, 33: 67-78.
14. Kader, A.A., W.J. Lipton and L.L. Morris, 1973. Systems for scoring quality of harvested Lettuce, *HortScience*, 8: 408-409.
15. McGuire, R.G., 1992. Reporting of objective color measurements. *HortScience*, 27: 1254-1255.
16. A.O.A.C., 2000. Association of Official Analytical Chemists. Washington DC. International 17th Edition, Revision I.
17. Ranganna, S., 1979. Manual of analysis of fruit and vegetable products. New Delhi India Tata Mc Graw-Hill Publishing Company, Limited Chapter, 4: 77-83.
18. Konopacka, D. and W.J. Plochanski, 2004. Effect of storage conditions on the relationship between apple firmness and texture acceptability. *Postharvest Biol. and Technol.*, 32: 205-211.
19. Marshall, S., 1992. Standard methods for examination of dairy products American public Health Association (ABHA). Washington DC, USA.
20. A.P.H.A., 1976. American Public Health Association. Compendium of methods the microbiological examination Foods, Washington. U.S.A.
21. Snedecor, G.W. and W.G. Cochran, 1989. Statistical Methods. 7th Ed., Iowa State Univ. Press. Ames. Iowa, U.S.A., pp: 593.
22. Ayranci, E. and S. Tunc, 2003. A method for the measurement of the oxygen permeability and the development of edible films to reduce the rate of oxidative reactions in fresh foods. *Food Chem.*, 80: 423-431.

23. Tanada-Palmu, P.S. and C.R.F. Grosso, 2005. Effect of edible wheat gluten-based films and coatings on refrigerated strawberry (*Fragaria ananassa*) quality. *Postharvest Biol. and Technol.*, 36: 199-208.
24. Shin, Y., J.A. Ryu, R.H. Liu, J.F. Nock and C.B. Watkins, 2008. Harvest maturity, storage temperature and relative humidity affect fruit quality, antioxidant contents and activity and inhibition of cell proliferation of strawberry fruit. *Postharvest Biol. and Technol.*, 49: 201-209.
25. Perkins-Veazie, P., 1995. Growth and ripening of strawberry fruits, *Hort. Rev.*, 17: 267-297.
26. Poovaiah, B.W., 1986. Role of calcium in prolonging storage life of fruits and vegetables. *Food Tech.*, 40: 86-89.
27. Colla, E., P.J.A. Sobral and F.C. Menegalli, 2006. *Amaranthus cruentus* flour edible films – influence of stearic acid addition, plasticizer concentration and the emulsion stirring speed on water vapor permeability and mechanical properties, *J. Agric. Food Chem.*, 54: 6645-6653.
28. Garcia, J.M., S. Herrera and A. Morilla, 1996. Effects of postharvest dips in calcium chloride on strawberry. *J. Agricultural and Food Chemistry*, 44: 30-33.
29. Holcroft, D.M. and A.A. Kader, 1999. Controlled atmosphere induced changes in pH and organic acid metabolism: My effect color of stored strawberry fruits. *Postharvest Biol. Technol.*, 17: 324-331.
30. Zeringue, H.I. and D. Bhatnagar, 1990. Inhibition of aflatoxin production in *Aspergillus flavus* infected cotton bolls after treatment with neem (*Azadirachta indica*) leaf extracts. *J. Amer. Oil Chem. Soc.*, 67: 215-216.
31. Rasooli, I. and M.R. Abyaneh, 2004. Inhibitory effect of thyme oils on growth and aflatoxin production by *Aspergillus parasiticus*. *Food Control*, 15: 479-483.
32. Shelef, L.A., 1994. Antimicrobial effects of lactates: a review. *J. Food Prot.*, 57: 445-450.
33. Weaver, R.A. and L.A. Shelef, 1993. Antilisterial activity of sodium, potassium or calcium lactate in pork liver sausage. *J. Food Safety*, 13: 133-146.