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ORIGINAL ARTICLE

Effect of microencapsulated essential oils on storage life and quality of strawberry (*Fragaria ananassa* cv. Camarosa)

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Abstract

Introduction Decay is an important factor that limits the storage life of strawberries after harvest and is responsible for significant economic losses. Control of postharvest decay in strawberry fruit has been mostly dependent on the use of fungicides. However, because of the negative effects of fungicides on the environment and human health, and the development of fungicide resistance by pathogens, there is an urgent need to seek alternatives. *Objectives* In this study the effect of *Rosmarinus officinalis* and *Thymus vulgaris* microencapsulated essential oils on shelf-life of strawberry var camarosa fruit was assayed. *Methods* Four different treatments were used: T1, control, none microencapsulation; T2, 0.2gr *Rosmarinus officinalis* oil microencapsul (ROM); T3, 0.2gr *Thymus vulgaris* oil microencapsul (TOM); T4, 0.1gr *Rosmarinus officinalis* oil microencapsul addition 0.1 gr *Thymus vulgaris* oil microencapsul (RTOM). *Results* All tested treatments indicated a significant delay in the change of weight loss, titrable acidity, total soluble solids, decaying percentage and firmness in strawberry fruits of experimental set than the control set. The significant impact of treatment is found on the least decay percentage in the order of fruits treated with T2, T3, T4, presumably because of the difference found in the release pattern of the oils could be due to the different hydrophilic characteristics. *Conclusion* Hence, it could be concluded that postharvest treatment with microencapsulated essential oils has the potential to control decaying incidence, prolong the storage life and preserve valuable attributes of postharvest strawberry, presumably because of its effect on inhibition of ripening and senescence processes and have antifungal activities.

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Introduction

Camarosa (*Fragaria ananassa* cv. Camarosa) is an important strawberry cultivar in all climates with mild winter (Hancock, 1999). This cultivar is also planted widely in Iran, especially near Jiroft. Strawberry is a very perishable fruit, usually with a very short shelf-life of 1–2 days when stored and transported at ambient temperatures. Decay is an important factor that limits the storage life of strawberries after harvest and is responsible for significant economic losses. Control of postharvest decay in strawberry fruit has been mostly dependent on the use of fungicides (Gil *et al.*, 1997).However, because of the negative effects of fungicides on the environment and human health, and the development of fungicide resistance by pathogens, there is an urgent need to seek alternatives.

Many essential oils and their constituents have antimicrobial activities, rendering these natural products good alternatives to synthetic fungicides (Adam *et al.*, 1998). Essential oils are classified as GRAS (generally regarded as safe) and

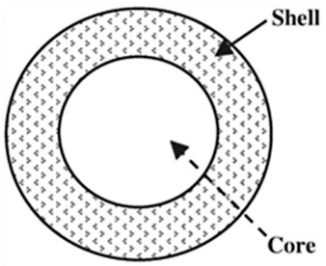

Figure 1 Schematic figure of microcapsule.

would therefore be more acceptable to consumers. The multicomponent nature of essential oils makes it more difficult for pathogens to build up resistance. The application of essential oils as alternatives, or in addition to synthetic fungicides, can contribute to prolonging the useful life of these synthetic agents in the postharvest environment. Many active agents such as essential oils are instable compounds. Microencapsulation technique can increase their stability and modify their release characteristics.

Microencapsulation cannot be defined as a product or as a component of a product. Rather, it is described as a process of enclosing micron-sized particles of solids or droplets of liquids or gasses in an inert shell, which in turn isolates and protects them from the external environment. The inertness is related to the reactivity of the shell with the core material. This technology is mainly used for the purpose of protection, controlled release and compatibility of the core materials. Although microencapsulation offers great potential in the coating industry, the resultant product of the microencapsulation process is termed a 'microcapsule'. Such capsules are of micrometer size (>1 μm) and have a spherical or irregular shape. Microcapsules can be divided into two parts, namely the core and the shell. The core (the intrinsic part) contains the active ingredient, while the shell (the extrinsic part) protects the core permanently or temporarily from the external atmosphere. A microcapsule is shown schematically in Figure 1.

Microcapsules have a number of interesting advantages, and the main reasons for microencapsulation can be summarized as follows:

- Protection of unstable, sensitive materials from their environments prior to use
- Better process ability (improving solubility, dispersibility and flow ability)
- Self-life enhancement by preventing degradative reactions (oxidation, dehydration)
- Controlled, sustained or timed release
- Safe and convenient handling of toxic materials
- Masking of odor or taste
- Enzyme and microorganism immobilization
- Controlled and targeted drug delivery
- Handling liquids as solids (Polk *et al.*, 1994).

The first systematic approach of phase separation – that is, partial desolvation of a homogeneous polymer solution into a polymer-rich phase (coacervate) and the poor polymer phase (coacervation medium) – was realized by Bungenberg and colleagues (Bungenberg de Jong & Kruyt, 1929; Bungenberg de Jong, 1949). These authors termed such a phase separation phenomenon 'coacervation'. The term originated from the Latin *acervus*, meaning 'heap'. This was the first reported process to be adapted for the industrial production of microcapsules. Currently, two methods for coacervation are available, namely simple and complex processes. The mechanism of microcapsule formation for both processes is identical, except for the way in which the phase separation is carried out. In simple coacervation, a desolvation agent is added for phase separation, whereas complex coacervation involves complexation between two oppositely charged polymers (Bungenberg de Jong & Kruyt, 1929; Bungenberg de Jong, 1949).

In order to apply the formulations with oil time release mechanisms to the fruits and vegetables preservative industry, the *Rosmarinus officinalis* and *Thymus vulgaris* oil microencapsulation were prepared by simple coacervation method by chitosan coating and β-cyclodextrin as coating material.

Chitosan is a modified natural carbohydrate polymer derived from chitin which has been found in a wide range of natural sources such as crustaceans, fungi, insects and some algae, and it is a well-known coating material used in several fruits for prolonging their shelf-life (Chien *et al.*, 2005).

In addition to protecting the core, the encapsulation provides controlled release essential oil. Therefore, antimicrobial activity of core from encapsulated powder is quite important for its application. The volatile release increased with the increase in water activity up to the point where collapse occurred such as chitosan. The effect of microencapsulation containing *R. officinalis* and *T. vulgaris* oil on decay and quality of fruits and vegetables are also important for its application. In this article, the antimicrobial properties of this two oil microencapsulation on the overall physico-chemical composition of Camarosa variety of strawberry during storage were studied. The main aim of the current research work was to check the way for shelf-life extension of the foresaid fruit.

Materials and methods

Essential oil extraction

Dry plant materials were distilled within 24 h in a steam distiller with an aqueous phase recycling system, using a plant material to water ratio of 2:1. The distillation time was about 2 h, and the oil obtained was separated from the aqueous solution and dried by treating with anhydrous Na_2SO_4. Each essential oil was transferred into a dark glass flask filled to the top and kept at a temperature of 4 °C until used.

Preparation of essential oils microencapsulation

The microencapsulation process was carried out by coacervation coupled with a vacuum-drying method described by Bhandari *et al.* (1998) and Ojagh *et al.* (2010), with minor modifications. An aqueous dispersion containing β-cyclodextrin (5 g) was dissolved in 200 mL of distilled water at 70 °C on a hot plate. After cooling to 40 °C, 0.5 mL of essential oil in ethanol (1:1 v/v) was slowly added to the solution with continuous agitation, to give a molar ratio of essential oil/β-CD of 0.4–2.4. The vessel was stirred for 3 h. Then the chitosan (1.0%), essential oil (0.15%), ethanol (20%), Tween 80 (0.2%) and glycerol (0.75%) were added. pH was regulated to 8 by the addition of a suitable amount of NaOH 1N and the complex solution was stirred by a magnetic stirrer at room temperature for 1 h. The hardened microparticles were filtered, rinsed with cold water and finally dried at 30 °C for 48 h under vacuum condition.

Selection of fruit and treatments and experimental design

For the present study, a local strawberry variety (Camarosa) was selected and ripened, uniform in size, color and weight; fresh fruit was obtained from the local market of Esfahan city. Essential oil microencapsulation was weighed and sealed in the synthetic package (4 cm × 5 cm), which were pricked evenly with a needle with two small holes before placement in the PE packaging. The weight of fruit for treatments was 100 ± 10 g. Four different treatments were used: T1, control, none microencapsulation; T2, 0.2 g *R. officinalis* oil microencapsul (ROM); T3, 0.2 g *T. vulgaris* oil microencapsul (TOM); T4, 0.1 g *R. officinalis* oil microencapsul + 0.1 g *T. vulgaris* oil microencapsul (RTOM). Treated samples and packaged microencapsulation were placed in PE film packaging [○, control (T1); □, T2; ◊, T3; △,T4], and they were stored for experimentation in the storage with their average maximum and minimum temperature at 5 ± 0.5 °C, and the effect of treatments after an interval of 3 days for 9 days of storage period was subjected to the following physicochemical analyses.

Sensory evaluation

The sensory evaluation of fruit was made by using hedonic nine-point scale for different characteristics such as peel color, flesh color, texture, taste and flavor by a panel of trained judges according to methods reported by Larmond (1977).

Decay or rotting (%)

The decay or rotting of the stored strawberry fruits were determined by their visual observations. Every fruit was sliced in five parts. Decay percentage of strawberry fruits was calculated as the number of decayed slice fruit divided by initial number of all slice fruits time × 100.

Physiological loss of weight (PLW) (%)

The PLW of mango fruit samples was calculated by considering the differences between initial weight and final weight of currently tested strawberry fruits divided by their initial weight.

% weight loss = weight of first interval – weight of second interval × 100 / weight of first interval

Titrable acidity and total soluble solid (TSS) of the sample

The titrable acidity (expressed as citric acid %) was determined by titrating 5 mL of juice with 0.1 N sodium hydroxide, using phenolphthalein as an indicator (AOAC, 1994). Acid % = 1/10 × Equivalent weight of acid × Titer × 100 / Weight of the sample.

The TSS content of the fruit was determined by using a refractometer (Atago Co., Tokyo, Japan). Homogenous sample was prepared by blending the strawberry flesh. The sample was thoroughly mixed and a few drops were taken on prism of the refractometer and direct reading was taken by reading the scale in meter as described in AOAC (1994).

Firmness determination

Fruit firmness was measured (kg cm^{-2}) at 1 or 2 points on the equatorial zone of strawberry for each treatmentby applying a plunger of 8 mm in diameter, using a penetometer FT 011 (TR Scientific Instruments, Forli, Italy).

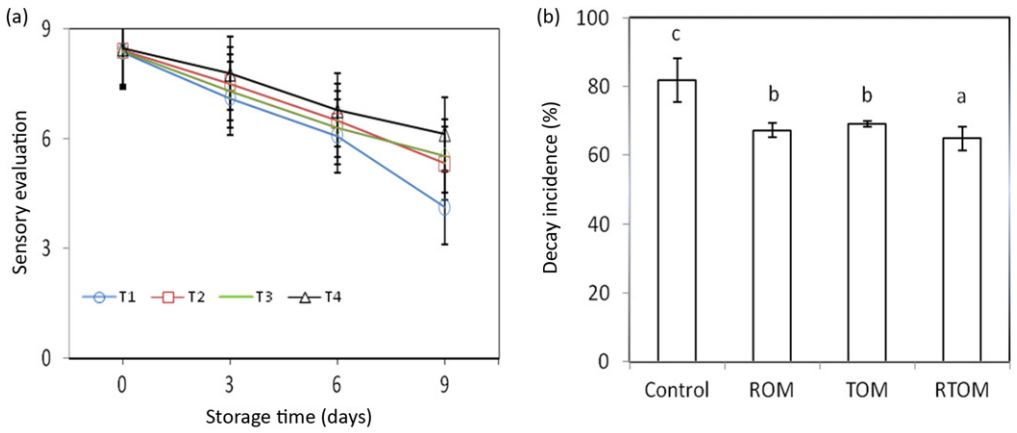

Figure 2 Effect of different treatments on decay incidence and sensory evaluation of strawberry variety Camarosa. T1, control; T2, *Rosmarinus officinalis* oil microencapsul (ROM); T3, *Thymus vulgaris* oil microencapsul (TOM); T4, *R officinalis* oil microencapsul and *T vulgaris* oil microencapsul (RTOM).

Statistical analysis

The experimental design was complete randomized design with three replicates. Analysis of variance was used to detect treatment effect and using the computer software SPSS version 15.0 (SPSS Inc., Chicago, IL, USA). Mean separation was performed by using least significance difference at the $P < 0.05$ level.

Results and discussion

Sensory evaluation and decay (%)

The statistical analysis showed that in general, the sensory evaluation was gradually decreased to 4.10 after 9 days of storage (Figure 2a). It might be due to fluctuations in acids, pH and sugar/acid ratio. Microcapsule with RTOM also received significantly highest score after 9 days of storage (6.13) while untreated control fruits had the lowest score (4.10). It might be due to the high decay incidence and the change in carbohydrates, proteins, amino acids, lipids and phenolic compounds that can influence the flavor, texture, taste and of fresh fruits.

Microencapsulated essential oil delayed the appearance of surface decay and kept good sensory acceptability in comparison with control strawberries (Figure 2b). The decay percentage was below 65% at the end of storage, whereas the control samples showed the highest decay incidence (81.77%). The result also suggests that two essential oils showed the antimicrobial properties. The oils of clove, oregano, rosemary, thyme, sage and vanillin have been found to be most consistently effective against microorganisms. They are generally more inhibitory against Gram-positive than against Gram-negative bacteria (Zaika, 1988). While this is true of many essential oils, there are some which are effective against both groups (oregano, clove, cinnamon and citral; Kim *et al.*, 1995; Sivropoulou *et al.*, 1996).

PLW

Generally, the weight loss of the strawberry fruit increases progressively during its storage. However, the weight of the currently tested fruits treated with the microcapsule is also found to have decreased, but in comparison with that of the fruits of the control set, the weight loss of microcapsule-treated fruits is found to be lesser. After 9 days of storage, the fruits of control set exhibited maximum weight loss (1.9%). That vapor–phase diffusion drived by a gradient of water vapor pressure at different locations is the reason for primary mechanism of moisture loss from fresh fruits and vegetables. A significant reduction ($P < 0.05$) in the weight loss by 1.23%, 1.36% and 1.42% was observed in the first three treatments of microcapsule (T4, T3 and T2, respectively) as compared with the control (Figure 3) possibly due to decreased in respiration rates. Previous experiments using eugenol, thymol or menthol vapors revealed benefits due to reduced weight loss in cherries and grapes (Martinez-Romero *et al.*, 2005; Serrano *et al.*, 2005).

Titrable acidity (TA) and TSS of the sample

The effect of different treatment on the content of TA and TSS in the strawberry samples was investigated as shown in Table 1. After 9 days of storage, TA content in the control samples was significantly lower than that of the initial

value. The untreated (control) fruits presented the highest decrease in TA while fruits treated with microencapsulation (T4) showed the lowest decrease after storage and TA was 6.01 mg per 100 g at day 9. Microencapsulated oil could inhibit TA loss by acting as an abiotic elicitor generating reactive oxygen species, which may also be due to the protection of antioxidant phenolics in essential oil (Hemeda & Klein, 1990).

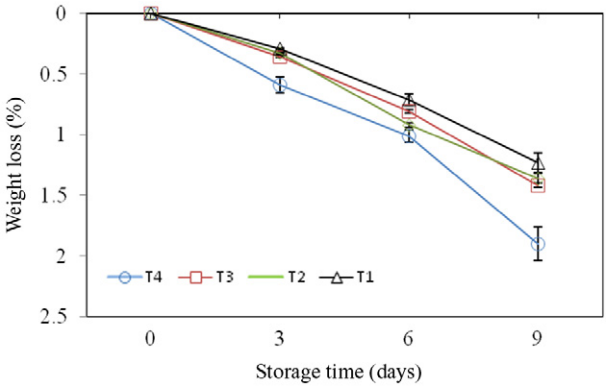

Figure 3 Effect of different treatments on physiological loss of weight (PLW) (%) of strawberry variety Camarosa.

Table 1 Effect of different microencapsulated essential oil on titrable acidity (TA) and total soluble solids (TSS) of the strawberry fruits var. Camarosa during storage period

	TSS (%)				TA (%)			
Treatments	0	3	6	9	0	3	6	9
T1 (Control)	6.81	7.41^{d}	7.12^{c}	7.43^{b}	6.66	7.32^{a}	6.00^{c}	5.01^{b}
T2 (ROM)		6.32^{a}	6.00^{a}	5.32^{a}		7.21^{a}	6.91^{a}	6.00^{a}
T3 (TOM)		6.27^{a}	6.08^{a}	5.29^{a}		7.30^{a}	6.34^{b}	5.98^{a}
T4 (RTOM)		6.50^{b}	6.20^{b}	5.35^{a}		7.21^{a}	6.33^{b}	6.01^{a}

Means with the same letters within a column are not significantly different at $P > 0.05$ using least significance difference.
Each value is the mean for three replicates.

The data presented in Table 1 show that a control sample had significantly the highest level of TSS value (7.43%) after 9 days of storage period. The TSS values of strawberry fruit treated with microcapsule treatments were lower than that of control samples. The reduction in the TSS of microcapsule-treated strawberry fruit was probably due to slowing down of respiration and metabolic activity, hence retarding the senescence process.

Firmness determination

The results associated with fruit firmness as influenced by microcapsule oils showed that maximum fruit firmness was retained in fruits treated with TOM (T3) (2 kg cm^{-2}) and minimum fruit firmness was noticed in untreated control fruits (T1). The data presented revealed that there was a similar decreasing trend in fruit firmness in all treatments toward the end of storage. Minimum firmness (1.6 kg cm^{-2}) was calculated after 9 days of storage while maximum (2 kg cm^{-2}), recorded at the first of storage (Figure 4). The ripening of strawberry fruits is characterized by a loss of firmness due to cell wall digestion by pectin esterase, polygalacturonase and other enzymes (Narain *et al.*, 1998). Previous reports on fruit firmness indicated that cherries and grapes were unaffected after exposure to eugenol, thymol or menthol vapors (Martinez-Romero *et al.*, 2005; Serrano *et al.*, 2005), while affected by cinnamon vapor exposure.

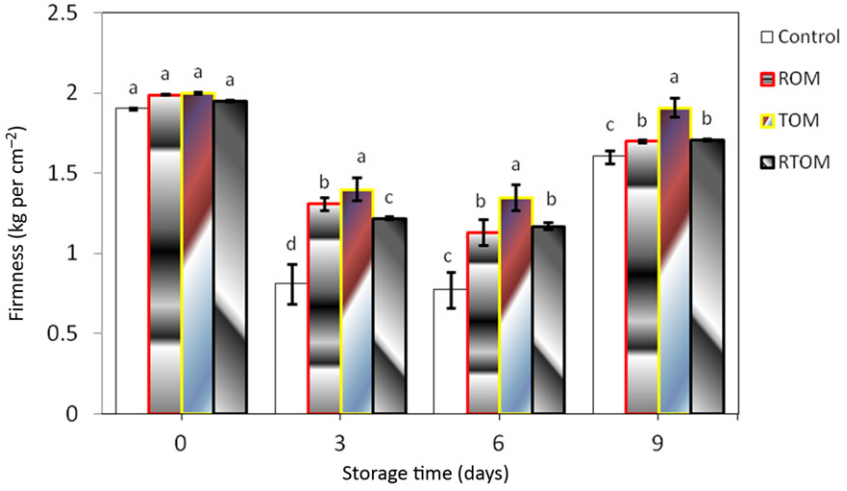

Figure 4 Effect of different treatments on firmness of strawberry variety Camarosa. Means with the same letters within each period of storage are not significantly different at $P > 0.05$ using LSD. ROM, *Rosmarinus officinalis* oil microencapsul; RTOM, *R. officinalis* oil microencapsul and *Thymus vulgaris* oil microencapsul; TOM, *Thymus vulgaris* oil microencapsul.

Pears (*Pyrus bertschneideri* L., cv. Reld), when treated with emulsions (3–9%) of commercial or refined plant oils at harvest and stored at 0 °C for 6 months, had maintained firmness in a concentration-dependent manner during storage (Ju *et al.*, 2000).

Conclusions

Thymus and *Rosmarinus* oils proved extremely effective on shelf-life of strawberry. The statistical differences between the control samples and treatments, carried out with formulations containing essential oils of different chemical composition, allow us to correlate the increase of shelf-life activity in the component of the oils. However, the toxicity of 1, 8 cineole, an active principle isolated from *Artemisa annua* essential oil, is known (Tripathi *et al.*, 2001), and so is the insecticidal and acaricidal activity of thymol (Karpouhtsis *et al.*, 1998). It can be asserted that the recorded toxic effect of each treatment is related to the synergetic action of the single components of the essential oil, as observed in recent studies. The methods used in the preparation process allowed the essential oils to be entrapped without any changes in their composition. The differences found in the release patterns could be due to the different hydrophilic characteristics of the examined oils. In fact, the high content of polar compounds in *Thymus* oil seems to favor the entrapment of aqueous phase into the microparticles during coacervation and subsequently a slower release. On the contrary, the greater content of little polar compounds, present in the essential *Rosmarinus* oil, could favor a more rapid release. This effect appears evident if the different amount of essential oil, as content in the microcapsules of *Thymus* and *Rosmarinus*, at the end of the test, is considered. This result suggests different applications of these formulations in the integrated shelf-life control strategies, particularly in the function of the chemical composition of the essential oils considered. The results obtained so far show that the encapsulation process is a suitable method for entrapping essential oils of a very different chemical composition. This method reduces loss of the active principles, leading to high-loaded microparticles that offer protection against environmental agents; it also offers the possibility of controlled release. They could be used to control the release of active principles in ways suitable for both acute and long-term treatments.

Given these encouraging results, further experiments are in progress to assess the suitability of natural active principle formulations for application as a new tool in the integrated control of shelf-life of different fruits.

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