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Optimization of Edible Coating Formulations on Zucchini to Reduce Water Loss

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ABSTRACT

*Zucchini (*Cucurbita pepo; melopepo*) fruit were coated with 0·5, 0·75 or 1·0% aqueous solutions of Semperfresh™ and with different formulations of calcium caseinate–acetylated monoglyceride aqueous emulsions ranging from 2·5 to 7·0% total solids. Semperfresh™ did not increase water vapor resistance of zucchini. Rates of respiration and ethylene production from coated and uncoated zucchini increased at the beginning of storage at 12·5 and 86% RH, but decreased rapidly thereafter. This type of response is indicative of a temporary metabolic disturbance resulting from preparation and a non-climacteric pattern of respiration. Coatings did not affect internal carbon dioxide or ethylene concentrations. Hue angle and lightness values were not significantly different for coated and uncoated zucchini. Ridge analysis indicated that a maximum water vapor resistance will result from relatively high sodium caseinate and low acetylated monoglyceride contents in edible coatings for zucchini fruit.*

INTRODUCTION

Zucchini or Italian squash (*Cucurbita pepo*; *melopepo*) is a fleshy immature fruit vegetable. It is characterized by a high respiration rate, a nonclimacteric pattern of respiration and high perishability. Zucchini fruit are very susceptible to water loss because they are picked at an early stage of growth with a poorly developed thin cuticle that is easily damaged during harvesting and subsequent handling. Injuries such as cuts, punctures, abrasions and scuffing, which damage the skin, are serious problems leading to abnormal cellular growth and microbial decay.

Immature fruits, such as zucchini, usually show less beneficial response to controlled atmospheres, because delay of ripening generally results in inferior quality (Kader *et al.*, 1989). The use of O_2 concentrations below 0·5% are not recommended for storage of zucchini, as this causes accumulation of exudate and increased susceptibility to decay (Leshuk & Saltveit, 1990). However, zucchini stored in 1% O_2 showed less chilling injury than those stored in air (Wang & Ji, 1989). Mencarelli *et al.* (1983) reported that reducing O_2 to about 2% helped minimize development of off-flavours in zucchini.

Films and coatings applied to fruit surfaces may reduce water loss, lower internal O_2 and increase internal CO_2 concentrations. They may also reduce respiration rate, retard ripening and alter the level of other physiologically active compounds (Trout *et al.*, 1952). The degree to which these factors can be altered for a given commodity will depend on species, cultivar, surface-to-volume ratio and respiration rate. The rates of water loss and respiration are dependent on maturity at harvest, source of produce, season and storage conditions. Traditionally, films and coatings have been used to reduce water loss, but new film materials and edible coatings formulated with a wider range of permeability characteristics facilitate achieving a 'modified atmosphere' effect in fresh fruits (Smith *et al.*, 1987).

Recent research by Krochta *et al.* (1990*a*, *b*) indicated that edible coatings made from milk protein and vegetable oil derivatives substantially reduced moisture loss. Also, films formulated with these two food components had different permeability values depending on the protein/lipid ratio (Avena-Bustillos & Krochta, 1993). Use of calcium caseinate as an emulsifier (Alanate-310™) makes possible the use at room temperature of an acetylated monoglyceride (Myvacet-5-07™), which melts at 48°C.

Pro-long™ or Semperfresh™, is a commercial coating formulated with sucrose esters of fatty acids, mono- and di-glycerides, and the sodium salt of carboxymethylcellulose. This coating has been applied successfully to retard ripening on bananas (Banks, 1984), apples (Drake *et al.*, 1987) and mangoes (Dhalla & Hanson, 1988), in aqueous solutions containing 1·5, 1·0 and 0·75% (w/v), respectively. Treatments at 1% resulted in increased ethanol formation in the pulp of some mangoes.

Response surface methodology (RSM) is a collection of mathematical and statistical techniques useful for analyzing problems in which several independent variables influence a dependent variable or response, and the goal is to optimize the response (Montgomery, 1984). For simplicity, the number of independent variables is usually limited to two or three. RSM is a powerful tool in experimental product development design (Dziezak, 1990) and has been applied in optimizing several food processing operations (Mudahar *et al.*, 1989).

The objectives of this study were to: (1) evaluate the effects of covering zucchini fruit with different edible emulsion coatings formulated with calcium caseinate and acetylated monoglyceride, and (2) compare the results obtained with those obtained with the commercial coating Semperfresh™. Factors of interest included resistance to water vapor diffusion, as well as respiration rate, ethylene production, internal carbon dioxide and ethylene concentration and surface color during storage of zucchini at 12·5°C and 86%. In addition, relationships between area, weight length and diameter of zucchini were developed. Routes of water loss in zucchini were also studied.

MATERIALS AND METHODS

Plant material

A preliminary experiment was done with zucchini fruit purchased from General Produce of Sacramento, CA, July, 1990. These fruits had been harvested in Watsonville, CA. Zucchini for optimization experiments were harvested directly by the researchers in August, 1990, at a farm in Greenfield, CA. Approximately 100 kg of fruit were harvested from six rows in the field, wrapped in brown paper towels to prevent mechanical damage and transported in open plastic boxes to the University of California, Davis. The zucchini were then placed in storage at 12·5°C and 86% RH, *c.* 8 h after harvesting. Next day the zucchini were sorted into three different sizes and stored again, unwrapped in open plastic boxes. After 2 days of preconditioning, zucchini were coated with different emulsion formulations following an experimental design to optimize coating formulation by response surface analysis (Montgomery, 1984).

Coating formulations

Semperfresh™, a food-grade coating used to retard moisture loss, ripening and spoilage of fruit, is a commercial product from INOTEK International (Mentor, OH). It is a mixture of sucrose esters with a high proportion of short chain unsaturated fatty acid esters, the sodium salt of carboxymethylcellulose, and mixed mono- and di-glycerides (Drake *et al.*, 1987). Solutions of 0·5, 0·75 and 1·0% Semperfresh™ were prepared by mixing with distilled water. Semperfresh™ powder was pre-wet by mixing to a slurry with *c.* 25% of total required water. After the slurry was completely mixed, the rest of the water was added and continuously mixed for 60 min at *c.* 20°C.

Alanate-310™, a calcium caseinate (CC) from New Zealand Milk Products (Santa Rosa, CA), was used to prepare emulsions with Myvacet-5-07™, distilled acetylated monoglycerides (AM) from Eastman Chemical Products (Kingsport, TN). These substances are food-grade materials, approved and used for coating of diverse food products. To prepare an emulsion, a solution of CC was first prepared by dissolving in distilled water at room temperature. The CC solution was heated to 50°C with constant stirring and then AM was added. After melting by heating to 60°C with constant stirring, the mixture was emulsified using a Polytron homogenizer (Brinkmann Instruments, Westbury, NY) at speed 5 for 60 s. Vacuum was then applied to remove air bubbles from the emulsion.

Edible coating formulations were evaluated in two steps, starting in the first step with a relatively high percentage of total solids in the CC–AM emulsions and recommended levels of Semperfresh™. Semperfresh™ coatings were not studied in the second step. The CC–AM emulsion coating formulations studied in both preliminary and optimization experiments are shown in Table 1. Semperfresh™ solutions and CC–AM emulsions were stable at 12·5°C for a week after formulation.

Water vapor resistance values were used as the response variable for optimization of edible coatings, because even small amounts of water loss usually results in flaccidity, shriveling and wrinkling of fruits. Furthermore, water vapor resistance was the parameter that showed most significant difference due to coating application on zucchini fruit.

Coating application

Coatings were applied using the CC–AM emulsions and Semperfresh™ solutions at room temperature. Initially, a 2·5 cm wide brush was used to spread the coatings on the fruit. The coatings were allowed to dry overnight in the storage room at 12·5°C and 95% RH, with the zucchini held in individual plastic weighing trays. Approximately 25 ml of Semperfresh™ solution and 35 ml of CC–AM emulsion were used to coat each kilogram of zucchini. The emulsion coatings had a milky or creamy

TABLE 1
Edible Coating Formulations for Zucchini Fruit

Code	*Total solids (%)*	*Alanate-310™ (CC) (%)*	*Myvacet 5-07™ (AM) (%)*
Preliminary experiment			
P1	2·5	0·5	2·0
P2	4·0	2·0	2·0
P3	4·0	1·0	3·0
P4	5·0	1·5	3·5
P5	5·0	0·5	4·5
P6	7·0	2·5	4·5
P7	0·5% Semperfresh		
P8	1·0% Semperfresh		
P9	1·5% Semperfresh		
P10	Control (uncoated)		
Optimization experiment			
T1	1·0	0·45	0·55
T2	1·0	0·30	0·70
T3	1·0	0·15	0·85
T4	1·5	0·68	0·82
T5	1·5	0·45	1·05
T6	1·5	0·22	1·28
T7	2·0	0·90	1·10
T8	2·0	0·60	1·40
T9	2·0	0·30	1·70
T10	Control (uncoated)		

appearance after application, but became transparent and practically invisible after drying. Semperfresh™ solutions were translucent and viscous, and formed practically invisible coatings after drying. Semperfresh™ coatings were slightly sticky, compared to CC–AM coatings and uncoated zucchini. Later, a study was done to evaluate the effect of applying the coatings by dipping. This was done because it was suspected that brushing could break the trichomes (skin surface hairs) of zucchini, thus aggravating the water loss. Dipping was accomplished by shaking individual fruits gently with a given CC–AM emulsion or Semperfresh™ solution formulation for 1 min in a plastic bag. A study was performed to estimate the effectiveness of coating using a tracer component in the CC–AM emulsion and Semperfresh™ solution formulations, because it was difficult to assess visually the completeness of a given coating application. The indicator used to assess uniform coating covering was 0·5% zinc silicate phosphor (Sigma, St. Louis, MO), which emitted fluorescent light under ultraviolet illumination.

Water vapor resistance

Coated and uncoated (control) zucchini were held on individual weighing trays sitting on shelves inside a storage room at 12·5 ± 1°C, 86 ± 4% RH and 3 m/s air flow. The zucchini were weighed with the weighing trays during the storage period. Weighing was done using a top-loading digital balance at 2-day intervals. Length and minimum and maximum diameter were measured intitially to calculate the zucchini area, assuming a cylindrical shape. Ten randomly picked fruits were used for each treatment and placed randomly on shelves in the constant temperature room. The weighing time was recorded to 1 min accuracy to calculate water vapor resistance. The route of water loss was studied by covering fruits completely or only on the stem or skin with petroleum jelly.

Response surface methodology

Based on response surface methodology, quantitative data were used to build empirical models to describe the relationships between the amount of CC and AM on the water vapor resistance of coated zucchini. The RSREG procedure from SAS/STAT (SAS, 1990*a*) was used to fit a second-order polynomial equation to experimental data. Three-dimensional surfaces, in which the levels of CC and AM were presented on horizontally perpendicular axes, and the response variable, water vapor resistance on the vertical axes, were generated using the G3D procedure from SAS/GRAPH (SAS, 1990*b*).

Respiration rate

A flow system was utilized for measurement of CO_2 and ethylene production during storage at 12·5°C. Three fruits with the same coating treatment having a total approximate weight of 500 g were placed in each 10 liter glass jar. The jars were sealed with a rubber stopper fitted with two plastic hoses for inlet and outlet of air. An air flow rate of 15 liter/h was selected to obtain a maximum rise in CO_2 concentration of 0·2% in the outlet air flow, assuming a maximum respiration rate of 60 ml of CO_2/kg h for zucchini at 12·5°C. The air flow rate was also selected to avoid water vapor condensation in the jars and subsequent increased fungus decay. Three jars were used for each of the nine treatments and for the control. Six 1-ml samples of outlet air were taken from each jar for triplicate measurements of CO_2 and ethylene every 2 days. Carbon dioxide concentration was measured using an infrared gas analyzer model PIR-2000 (Horiba Instruments, Irvine, CA), and the ethylene concentration was measured using an AGC-211 Hach Carle analytical gas chromatograph (Hach, Loveland, CO), with a flame ionization detector.

Internal gas composition

For extraction and analysis of internal gas composition, a procedure recommended by Saltveit (1982) was used. The procedure involved use of a 20-liter Plexiglas vacuum chamber filled to 90% capacity with a degassed saturated solution of magnesium sulfate (Epsom salt, $MgSO_4.7H_2O$, with a solubility of 71 g/100 ml cold water). The saturated solution was acidified to pH 2·5 with HCl to reduce CO_2 solubility. Degassing of the solution was done by exposure to vaccum for several hours before use. Two zucchini fruits were submerged in the degassed saturated solution and put inside a submerged inverted glass funnel whose tip was covered tightly with a rubber septum. A partial vacuum (300 mm Hg) was then applied. Gas bubbling out of the zucchini rose and accumulated at the top of the inverted funnel. The vacuum was released after 10 min and then six 1-ml gas samples were collected for CO_2 and ethylene measurements.

Color

Color changes during storage were evaluated using a Minolta Chroma Meter CR200 (Minolta Camera, Japan) by determining L^*, a^* and b^* parameters after calibration with a standard green tile. The L^* parameter was used to indicate lightness value, and the a^* and b^* parameters were used to calculate the hue angle Θ, $\tan^{-1}(b^*/a^*)$, according to Little (1976). Five fruits were used each time, with color measurements taken on areas close to the blossom end, stem end and middle section of each fruit.

RESULTS AND DISCUSSION

Effects of coating application

Visual examination under ultraviolet light showed that emulsion and solution coatings completely covered the zucchini surface, as evidenced by uniform fluorescence. A significant difference in water loss, depending on the coating application method, was found. Dipping was 20% more effective than brushing in reducing water loss. It is known that trichomes, which can be removed by mechanical abrasion, help to avoid water loss by reducing air flow on the evaporating surface and trapping water vapor (Kader *et al.*, 1989). Removal of trichomes also leaves wounds, which allow greater water loss. Thus, brushing may have removed trichomes.

By selective coating of fruit with petroleum jelly, it was found that 78% of water loss was through the skin and 22% was through the stem end. Stem end loss was quite large, considering that the stem end is a relatively small portion of the surface area of zucchini fruit. Excessive coating of zucchini, as demonstrated with complete coverage with petroleum jelly, could induce anaerobiosis and cell wall pectin hydrolysis.

Water vapor resistance

Water loss was estimated by subtracting the weight measurements taken at different times from the initial weight. The contribution of respiration rate to weight loss was neglected in this calculation. Simple linear regression analysis of weight loss versus time was performed for five replicates for each treatment, and the slope of the line was calculated using Lotus 1-2-3 (Cassel, 1986). The correlation coefficient was also calculated. Water loss was found to follow a linear relationship at $p < 0{\cdot}001$, and its slope in g/h provided the rate of water loss.

An estimate of water vapor resistance was calculated using a modified Fick's equation (Ben-Yehoshua *et al.*, 1985):

$$r = \left[\frac{\left(A_W - \frac{\%RH}{100}\right) P_{WV}}{RT}\right] \left(\frac{A}{J}\right)$$

where:

r = water vapor resistance (s/cm)
A_W = water activity of zucchini (0·996) (Chirife & Ferro Fontan, 1982)
%RH = storage room relative humidity (86% RH)
P_{WV} = saturated water vapor pressure at 12·5°C (10·87 mm Hg)
R = universal gas constant (3464·629 mm Hg cm^3/g K)
T = storage room temperature (285·5 K (12·5°C))
A = surface area of zucchini (cm^2)
J = slope of water loss in zucchini vs. storage time (g/s)

In order to compare the relative rates of water loss, it was necessary to account for the different exposed areas of zucchini fruit. To accomplish this, 10 fruits were peeled and the peel contour was drawn on paper. The drawn surface area was measured with a planimeter to develop linear correlations with initial weight and calculated area, assuming cylindrical shape for zucchini according to the formula $A = \pi D(h + D/2)$. D and h, fruit diameter and length, respectively, were measured with a caliper. The linear regression equations and coefficients of correlation for noncylindrically shaped zucchini are:

Area = 77·65 + 0·70 (calculated area); $r = 0{\cdot}88$, $p < 0{\cdot}001$, range = 290–480 cm^2

Area = 139·89 + 0·62 (initial weight); $r = 0{\cdot}98$, $p < 0{\cdot}001$, range = 230–460 g

For cylindrically shaped zucchini the linear regression equations and coefficients of correlation are:

Area = 37·58 + 0·61 (calculated area); $r = 0{\cdot}86$, $p < 0{\cdot}001$, range = 130–190 cm^2

Area = 56·68 + 0·83 (initial weight), $r = 0{\cdot}81$, $p < 0{\cdot}01$, range = 80–120 g

It was concluded that a correlation of surface area with zucchini weight produced a better fit generally than using the assumption of a right cylinder formula for area estimation for noncylindrically shaped zucchini. However, for cylindrically shaped zucchini, the right cylinder assumption fitted better than using initial fruit weight.

One-factor, completely randomized analysis of variance and Duncan's multiple range test of the preliminary experiment indicated that there was not a significant difference in water vapor resistance among the uncoated, Semperfresh™-coated and most of the CC–AM coated zucchini, as shown in Fig. 1. However, the 1·0% CC/3·0% AM, 0·5% CC/4·5% AM and 2·5% CC/4·5% AM coatings had higher water vapor resistance than the uncoated zucchini. Motlaugh and Quantic (1988) reported no differences in water loss of limes coated with 1·5, 2·0 and 2·5% Semperfresh™. Similarly, Smith and Stow (1984) reported a nonsignificant reduction in water loss during storage of apples, using a 1 to 4% Semperfresh™ coating. They reported that the most marked effect of Semperfresh™ coating was an increase in internal CO_2 concentration. Also, Dhalla and Hanson (1988) reported that 1·0% Semperfresh™ increased ethanol formation in the pulp of some mangoes, while 0·75% Semperfresh™ coating significantly increased the shelf-life of this fruit.

Examination of the CC–AM data from the preliminary experiment, using response surface analysis (Montgomery, 1984) (Fig. 2), pointed to

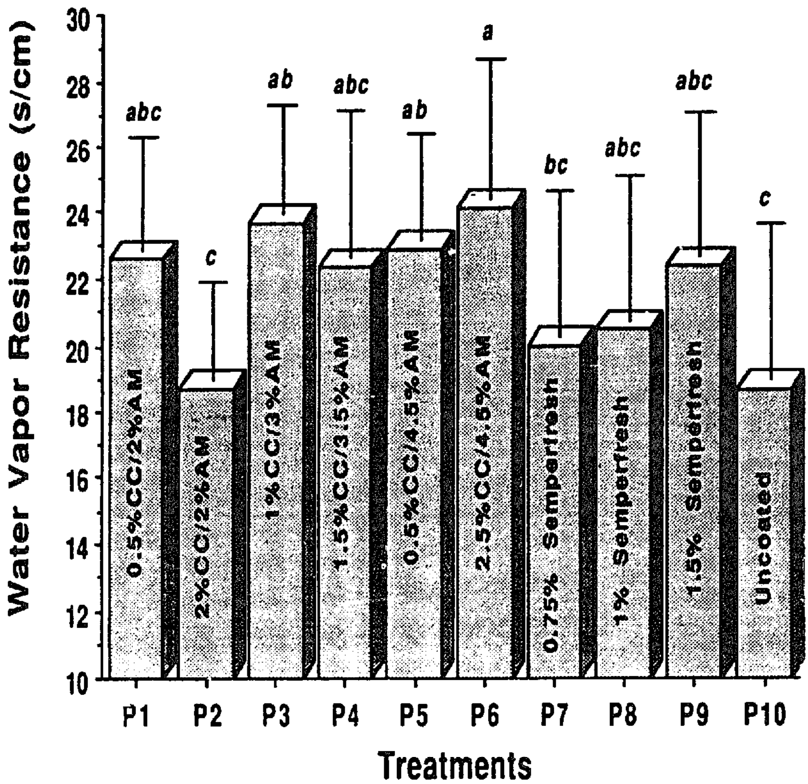

Fig. 1. Water vapor resistance of coated and uncoated zucchini fruit stored at 12·5°C and 86% RH. Letters indicate significant difference at $p < 0{\cdot}05$. (Preliminary experiments).

reduced levels of percentage total solids in emulsions and a higher proportion of AM in the emulsions. Thus, an optimization experiment was designed in the second step to explore CC–AM emulsion coating formulations, using lower total solids.

In the optimization experiment, levels of water loss during the storage period of 20 days were as high as 14·3% for uncoated zucchini and as low as 7·9% for zucchini coated with the best CC–AM formulation. From one-factor, completely randomized analysis of variance and Duncan's multiple range test of water vapor resistance of the different treatments, it was concluded that all coating treatments had significantly higher water vapor resistance than the control at $p < 0{\cdot}05$, as shown in Fig. 3. The CC–AM coating treatments were not significantly different from each other, except for the 0·15% CC/0·85% AM (T3) treatment, which gave the highest water loss among the coatings. These results demonstrate that it is possible to increase by 75% the water vapor resistance of zucchini with a 2% total solids emulsion formulated as 0·9% CC/1·1% AM (T7).

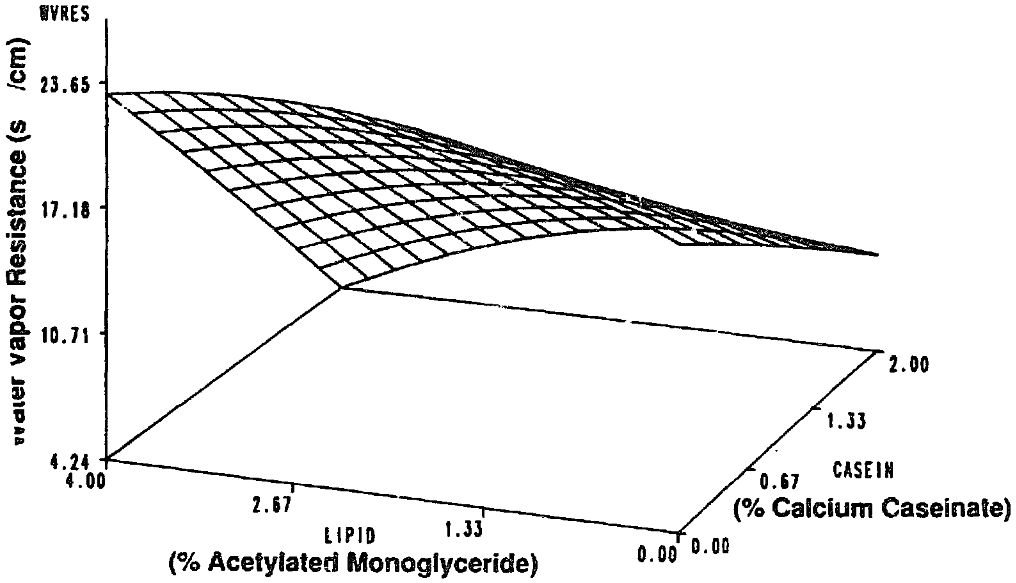

Fig. 2. Preliminary water vapor resistance optimization for coated zucchini fruit.

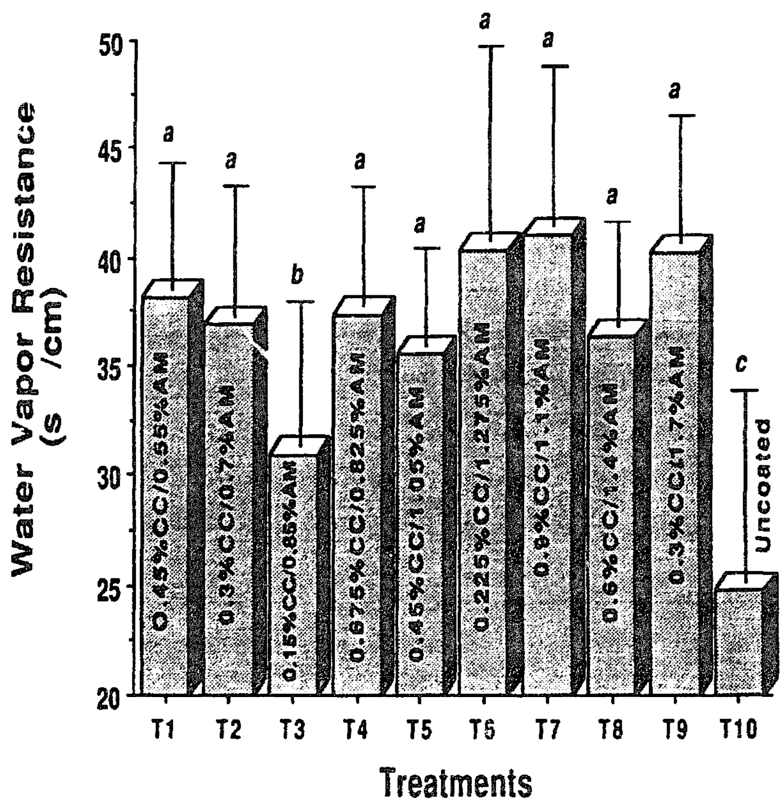

Fig. 3. Water vapor resistance of coated and uncoated zucchini fruit stored at 12·5°C and 86% RH. Letters indicate significant difference at $p < 0{\cdot}5$. (Optimization study.)

Response surface analysis of water vapor resistance indicated that vapor resistance decreased as coatings with high concentrations of total solids were used, as is shown in Fig. 4. By canonical analysis (SAS, 1990*b*), it was found that a saddle-type surface ridge indicated that maximum water vapor resistance could be achieved by decreasing the total solids concentration in the emulsion coating and reducing the amount of AM relative to caseinates.

Respiration rate

Control and coated zucchini all followed a decreasing, nonclimacteric respiration pattern (Fig. 5). By one-factor, completely randomized analysis of variance, the respiration rate was determined to be significantly higher at $p < 0{\cdot}05$ at the start of the experiment for most coated zucchini (only coating T7 shown) compared to the control. Only coating T3 (0·15% CC/0·85% AM) produced a significantly lower respiration rate. These differences were probably due to temporary metabolic disturbances caused by the coatings. The respiration rate decreased from 55 to 20 ml of CO_2/kg h for uncoated zucchini after 16 days. This final respiration value was essentially the same for coated and uncoated zucchini and indicated that coating did not permanently modify respiration. The low respiration rate is consistent with the long storage life for zucchini in this study. Coated and uncoated zucchini were in good eating condition for up to 50 days of storage. Mencarelli *et al.* (1983) reported a respira-

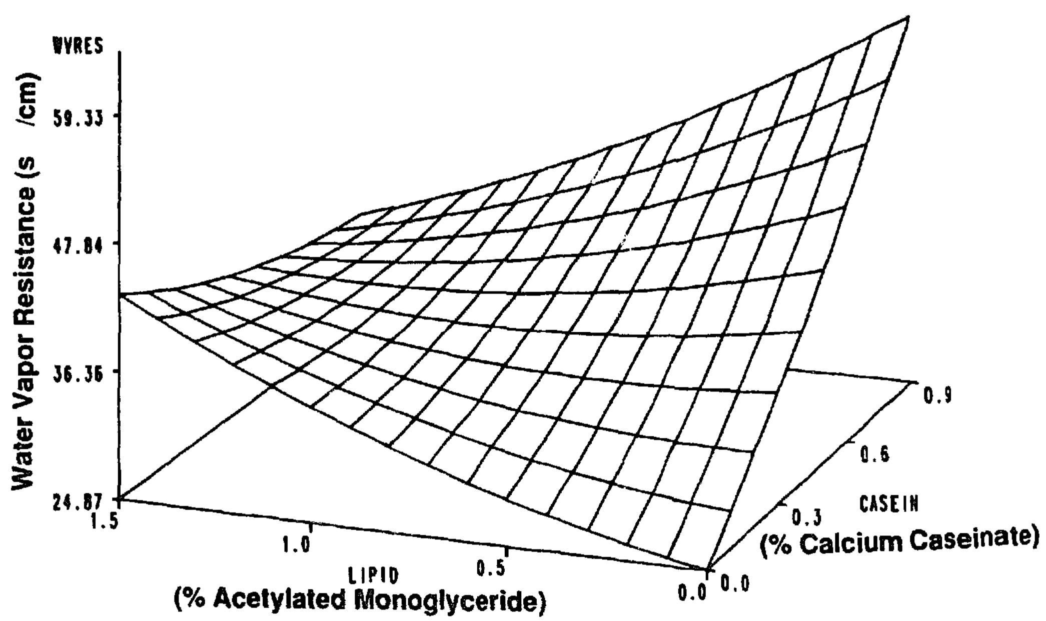

Fig. 4. Water vapor resistance optimization for coated zucchini fruit.

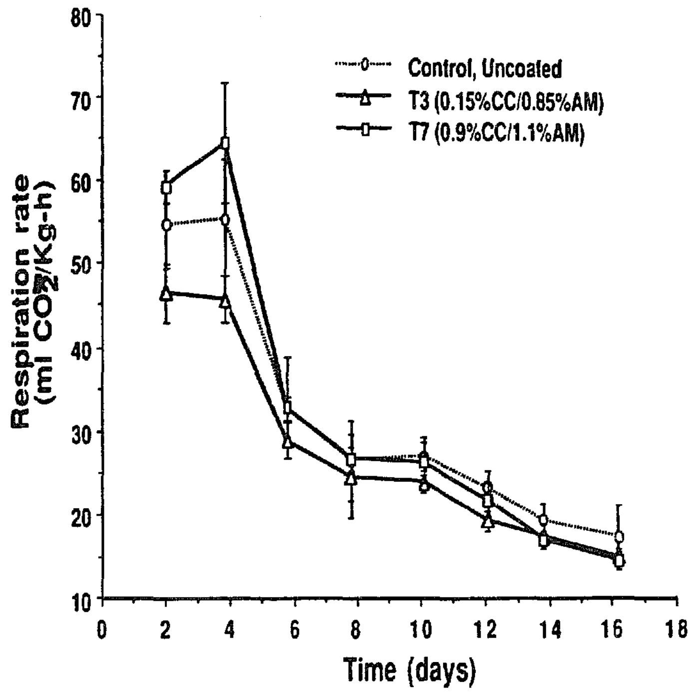

Fig. 5. Respiration rate of coated and uncoated zucchini fruit stored at 12·5°C.

tion rate decrease from 100 to 60 ml of CO_2/kg-h in a storage period of 14 days at 10°C. Differences between the Mencarelli results and our data could be due to zucchini varietal and maturity effects.

After applying one-factor, completely randomized analysis of variance, it was concluded that ethylene produced by the coated and uncoated zucchini was not significantly different during the storage period, and ethylene production decreased at the end of the storage time for all treatments (Fig. 6). Mencarelli *et al.* (1983) reported a nearly constant production of 1·25 μl/kg h in zucchini stored for the same length of time. Again, differences could be due to zucchini varietal and maturity effects. During preliminary respiration rate experiments in glass jars, accumulation of exudate and water condensation on coated zucchini hydrated and dissolved the CC–AM coatings. This resulted in a marked increased susceptibility of these zucchini to mold growth and rapid decay. This problem was not observed in similar samples stored outside glass jars and when the air flow rate was sufficiently high to avoid water condensation.

Internal gas concentration

Internal CO_2 concentration decreased during storage without a significant difference between the control and coated zucchini (Fig. 7). Internal

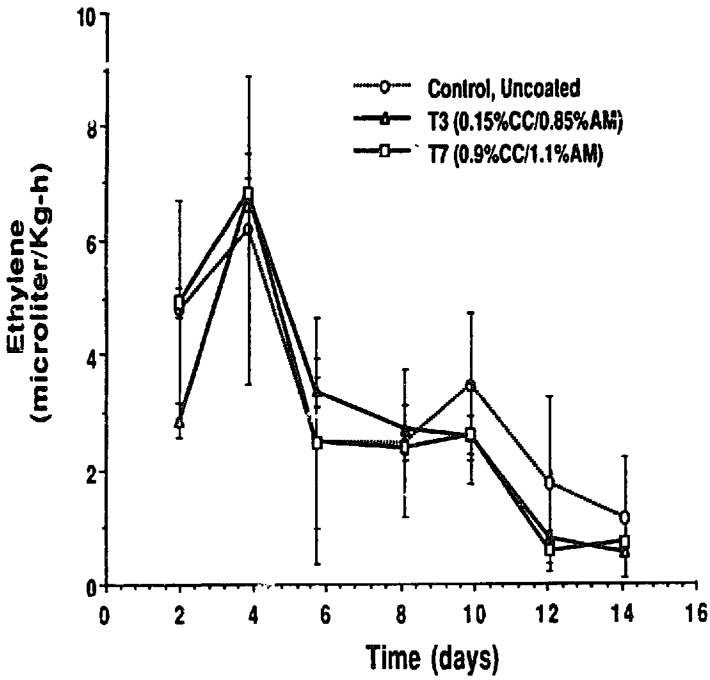

Fig. 6. Ethylene production rate of coated and uncoated zucchini fruit stored at 12·5°C.

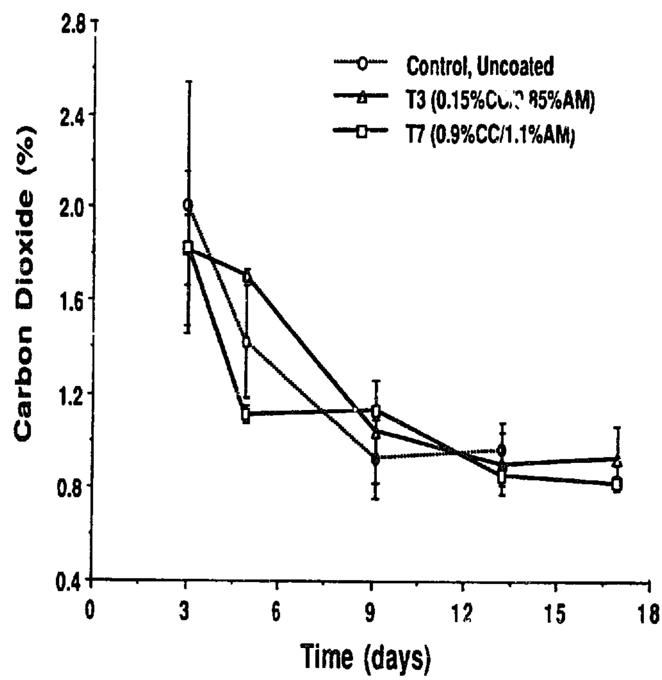

Fig. 7. Internal CO_2 in coated and uncoated zucchini fruit stored at 12·5°C.

ethylene concentrations were also not significantly different for coated and uncoated zucchini at the beginning of storage, and declined slightly in both coated and uncoated zucchini towards the end of storage (Fig. 8).

Color

From one-factor, completely randomized analysis of variance, it was concluded that there was not a significant difference in hue angle (Fig. 9) or lightness (Fig. 10) between uncoated and coated zucchini. Also, hue angle did not decrease significantly during storage of coated and uncoated zucchini.

CONCLUSIONS

Semperfresh™ did not increase water vapor resistance of zucchini fruit. The emulsifying properties of CC facilitated the incorporation of high melting AM in emulsion coatings. Uniform surface covering was enhanced by the surfactant properties of CC, resulting in increased water vapor resistance at relatively high caseinate content in emulsion coatings.

Response surface methodology is a useful tool for optimizing emulsion coating formulations. Ridge analysis indicated that a maximum

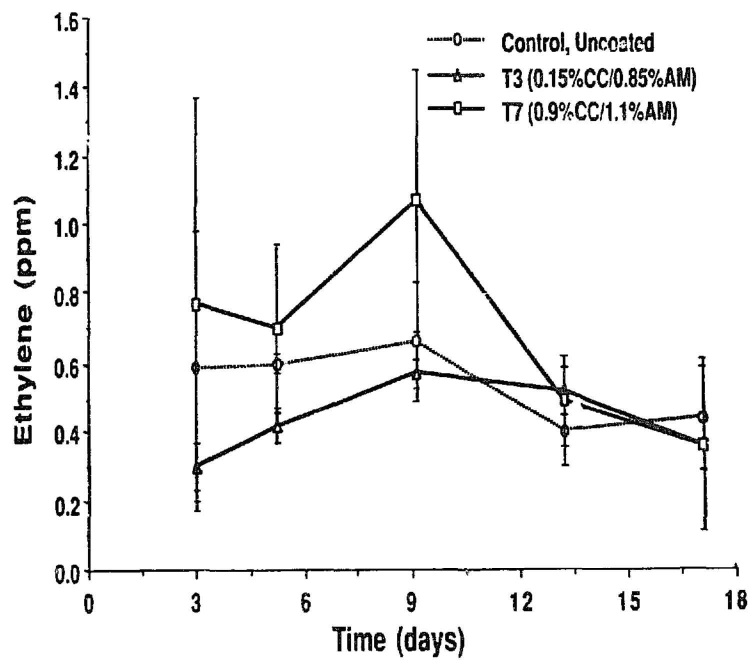

Fig. 8. Internal ethylene concentration in coated and uncoated zucchini fruit stored at 12·5°C.

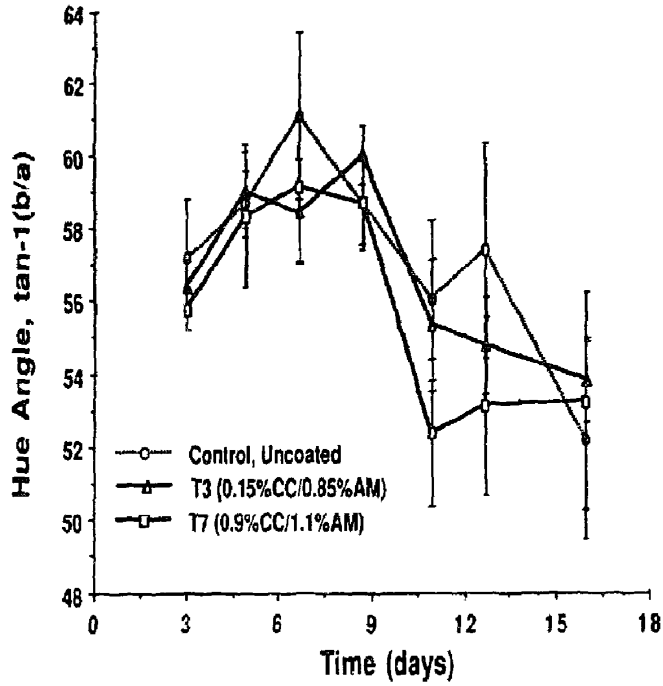

Fig. 9. Color or hue angle (tan 1(b/a)) of coated and uncoated zucchini fruit stored at 12·5°C.

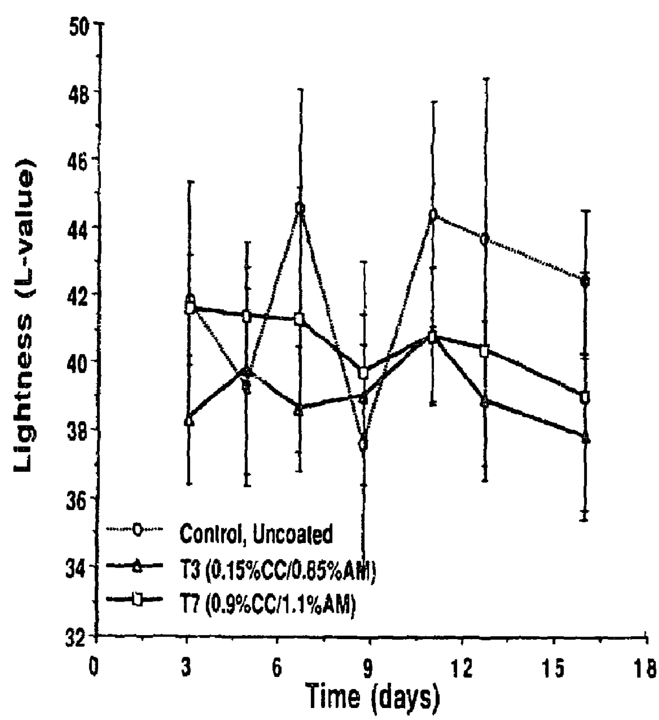

Fig. 10. Lightness of L value for coated and uncoated zucchini fruit stored at 12·5°C.

water vapor resistance will result from relatively high CC and low AM content in edible coatings for zucchini.

Respiration rate and ethylene production rate in coated and uncoated zucchini increased at the beginning of storage at 12·5°C and 86% RH, but decreased rapidly as evidence of temporary metabolic disturbance during application of coatings and a nonclimatic pattern of respiration. Coatings did not affect internal CO_2 and ethylene concentrations. Hue angle and lightness values were not significantly different for coated and uncoated zucchini. Beneficial effects of edible coatings are mainly a consequence of increased water vapor resistance (up to 75%) in zucchini fruit.

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