Evolution of Selected Volatiles in Chitosan-Coated Strawberries (*Fragaria* × *ananassa*) during Refrigerated Storage

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The effect of chitosan coating on the evolution of several volatile compounds relevant to the strawberry (*Fragaria* × *ananassa* cv. Camarosa) aroma profile has been investigated. Strawberries dipped in chitosan acetate solution at 1 or 1.5% (w/w) and uncoated controls were stored at 10 °C for 1 week. Significant differences in aroma profile between coated and uncoated samples were observed. Most importantly, the buildup of the off-flavors acetaldehyde and ethanol was largely delayed in coated berries. With regard to the effect of chitosan on ester evolution, the levels of ethyl butanoate and ethyl hexanoate, important contributors to strawberry aroma related to fruity and sweet notes, were found to be enhanced in coated fruit. Acetate esters also increased during storage but more markedly in uncoated strawberries. These results show the potential of chitosan coatings in maintaining strawberry flavor during storage, something difficult to achieve with current conservation technologies. Moreover, differences in results for different coating concentrations are reported.

KEYWORDS: Strawberry (*Fragaria* × *ananassa*); chitosan coating; aroma profile; off-flavors; storage

INTRODUCTION

The typical aroma of strawberry fruit is one of the most appreciated around the world. It is the result of a balance between different volatile molecules including esters, alcohols, carbonyls, and furanones, where the most important general components are ethyl hexanoate, ethyl butanoate, dimethyl-4-hydroxy-3(2*H*)-furanone, methyl butanoate, and methyl hexanoate (*1–3*). Many other volatiles, even though they are present in low concentrations, are important cultivar-specific aroma compounds. Nevertheless, unwanted changes in this delicate balance can occur during the postharvest period. Such changes result from losses of desirable aroma compounds as well as the development of off-flavors. Several conservation technologies such as controlled atmosphere (CA) and modified atmosphere packaging (MAP) have been developed to prolong strawberry shelf life. However, changes in the aroma profile have been shown to persist, mainly because of the development of off-flavors (*4, 5*).

Edible coatings have long been known to extend the shelf life of fresh fruits and vegetables by means of modifying the respiration rate and reducing water loss. Edible coatings can also prevent mechanical injury produced during postharvest handling and processing and improve appearance. Edible coatings comprise polysaccharides, proteins, and lipids, which can be combined to improve their barrier properties, integrity, adhesivity, and flexibility. Among such coatings, chitosan, a polysaccharide obtained as a byproduct of the seafood industry or produced by some fungi (*Aspergillus niger*, *Mucor rouxii*, *Penicillium notatum*) has antimicrobial activity as well as excellent film-forming characteristics. Chitosan-based coatings delay fungal growth and senescence in strawberries (*6*). This polymer has been described as an ideal preservative for fresh berries.

The capacity of a polymer coating to modify the internal atmosphere of fruit and retain volatile aroma compounds may affect flavor. Coating formulations excessively restricting the exchange of CO_2 and O_2 between fruit and the environment lead to the formation and accumulation of anaerobic volatiles such as ethanol and acetaldehyde and the development of undesirable flavor changes. However, edible coatings capable of providing gas exchange without reaching anaerobiosis and restricting the loss of volatile organic compounds could enhance fruit flavor. Wax coatings have been reported to cause a buildup of ethanol and acetaldehyde and alter citrus fruit flavor. Fruits coated with shellac are more prone to the buildup of ethanol and acetaldehyde as well as large flavor differences compared to those covered with more permeable coatings consisting of carnauba- and polyethylene-based waxes (*7*) or polysaccharide-type coatings based on a cellulose derivative (*8*). A coating of mango carnauba improved aroma biosynthesis in mango fruit (*9*). However, in the same study Semprefresh coating (sucrose esters of fatty acids, sodium carboxymethyl cellulose, and

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monodiglycerides of fatty acids) and aloe vera gel coating reduced aroma development.

Exposure to high CO_2 and/or low O_2 atmospheres has been shown to modify the aroma profile of strawberries, which might have a detrimental effect on their final sensory quality. Considerable work has been done with regard to the effect of CO_2-enriched atmospheres on the flavor quality of strawberries. Elevated CO_2 atmospheres favor the accumulation of fermentative metabolites, which can cause off-flavors at high concentrations (*4, 5, 10–14*). Ethanol accumulation has been associated with changes in the volatile aroma profile of strawberries due to an increase in the production of ethyl esters over other alkyl esters and a reduction in the concentration of acetate esters other than ethyl acetate (*13–15*).

There are reports in the literature regarding the capacity of chitosan to modify the internal atmosphere of fresh commodities. It has been observed that chitosan coatings decrease O_2 levels and raise CO_2 levels within tomato (*16*), apple (*17*), and Japanese pear (*18*) without reaching anaerobiosis and with internal CO_2 levels of no more than 10%. According to these papers, no ethanol flavor was detected in apples and pears, the latter being significantly better tasting than the control fruit. Whereas the effects of chitosan on the postharvest life of strawberry fruit have been widely investigated, no work has been done to quantify the evolution of this fruit's volatile aroma profile, a very important parameter of fruit quality and marketability.

In a previous study (*6*), it was shown that chitosan coating applied at 1 and 1.5% (w/w) on strawberries stored over 1 week at 10 °C delayed senescence and fungal growth and reduced respiration rate, and the treated fruit presented a better visual appearance than uncoated fruit (*6*). In view of the positive effects of chitosan coating on fruit quality, the aim of this work has been to study the evolution of selected volatile compounds in strawberries coated with chitosan during 1 week of storage at 10 °C.

MATERIALS AND METHODS

Plant Material. Commercially grown strawberries (*Fragaria × ananassa* Duch. cv Camarosa) from Palos de la Frontera (Huelva, Spain; longitude, 37° 14′ N; latitude, 6° 53′ W) were picked during the third week of May. After harvesting, strawberries were shipped in a refrigerated truck to Valencia and stored at 2 °C overnight until used in experiments. The next morning three batches of strawberries of uniform size were selected, requiring at least two-thirds of their surface to be of red color and free of physical damage or fungal infection.

Chemicals. Reference compounds were purchased from Sigma-Aldrich Corp. (St. Louis, MO) and used in gas chromatography for compound identification and calibration.

Edible Coating Formulations. Acetic acid and high molecular weight chitosan were purchased from Sigma-Aldrich Corp. Coating solutions were prepared by dissolving 1% chitosan in 0.25% acetic acid solution and 1.5% chitosan in 0.50% acetic acid solution. The final pH of the coating solutions was 5.5.

Sample Preparation. Strawberries were randomly distributed into three groups of 375 each. One group was used as the control, and the other two groups were assigned to treatments with 1 or 1.5% chitosan solutions. Strawberries were immersed for 5 min in 1 or 1.5% chitosan solution and were allowed to dry for 2 h at room temperature and 50% relative humidity. Strawberries were stored in perforated plastic punnets, each containing 25 fruits at 10 °C and 70–80% relative humidity in a refrigerator simulating conditions of storage at home after consumer purchase. After 0, 2, 4, 6, and 7 days of storage, three punnets per treatment were randomly removed from the refrigerator, and 10 strawberries free of fungal infection from each punnet were blended

in a conventional blender. Two and a half grams of the obtained purée was placed in a 10 mL glass vial, crimp-sealed, and frozen at −20 °C until analysis within the next month.

Identification and Quantification of Volatile Compounds. All volatiles, 3 fermentative metabolites (acetaldehyde, ethanol, and ethyl acetate), and 16 typical strawberry flavor compounds were identified by GC-MS and quantified by GC. Frozen strawberry purée was thawed at room temperature and subsequently heated to 50 °C for 20 min in a conventional oven. A 65 μm PDMS/DVB SPME fiber (Supelco Inc., Barcelona, Spain) was exposed to the vial headspace for 20 min, and the trapped volatiles were immediately desorbed (for 5 min) at the splitless injection port of a GC Hewlett-Packard 5890 series II (Agilent Technology, Barcelona, Spain) equipped with a FID and an Rtx-1301 column (0.50 μm × 0.53 mm × 30 m, Restek, Teknokroma, Barcelona, Spain). The oven was first kept at an initial temperature of 40 °C for 5 min; this was increased at a rate of 5 °C/min to 200 °C and then maintained for 2 min. The injector and detector temperatures were 240 °C. Three vials per treatment were analyzed. Previous compound identification was performed by GC-MS with an HP 5890 series II gas chromatograph equipped with an HP 5972 mass selective detector (Hewlett-Packard, Palo Alto, CA) using the same column and chromatographic conditions. The GC–mass spectrometer interface was maintained at 240 °C. Mass spectra were obtained by electron impact at 70 eV, and data were acquired across the range of 29–400 μm. GC-MS and GC-FID peaks were correlated using pure compounds.

Because the amount of volatile compounds gained by the SPME fiber is dependent on the food matrix composition, quantification was performed after calibration of the GC-SPME system by the standard addition method. Two and 6 μL of the pure compounds (Microliter Model 701 Hamilton syringe, Bonaduz, Switzerland) were added to 6 g of strawberry purée samples and stirred. Dilutions of 1/10 and 1/100 (measured by weight) were obtained from these samples. The resulting six samples (containing the pure compound in the range of 1–1000 μL/kg of strawberry purée) were then frozen and stored at −20 °C until analyzed following the procedure already described. Samples were prepared in triplicate and analyzed three times. All calibration curves presented good linearity; the correlation coefficients were all > 0.998. The initial concentrations of the diverse compounds in the strawberry purée were determined by extrapolating the standard addition calibration curve to the *x* intercept.

Statistical Analysis. The StatGraphics Plus program, version 2.1 (Statistical Graphics Corp.), was used for analysis of variance (ANOVA) and to test significant differences between mean values at $p \leq 0.05$.

RESULTS AND DISCUSSION

The evolution of the selected volatiles for samples of strawberries coated with chitosan acetate solutions at 1 and 1.5% (w/w) and that of untreated controls are described below. All samples were stored for 7 days at 10 °C, and aroma volatiles were analyzed at days 0, 2, 4, 6, and 7.

Fermentative Metabolites. Acetaldehyde is a naturally occurring volatile in almost all fruits and accumulates during ripening. It is a precursor of the ethyl esters produced during maturation of strawberries (*19*). In the anaerobic pathway, acetaldehyde is produced via pyruvate decarboxylation by the enzyme pyruvate decarboxylase. Acetaldehyde may also be metabolized to ethanol by the enzyme alcohol dehygrogenase and to acetyl CoA by the enzyme aldehyde dehydrogenase. Ethanol and acetyl CoA are substrates for the synthesis of ethyl acetate. Acetaldehyde, ethanol, and ethyl acetate are produced by strawberries when stored under aerobic conditions. Fruit senescence increases anaerobic respiration and the levels of acetaldehyde and ethanol. Postharvest treatments with low O_2 and/or high CO_2 atmospheres induce the accumulation of acetaldehyde, ethanol, and ethyl acetate. Strawberries kept in low O_2 (in N_2) atmospheres accumulate lower levels of ethanol and acetaldehyde than those stored under elevated CO_2 levels (20–80% in air) (*10*). In strawberries stored at 0 °C in a range
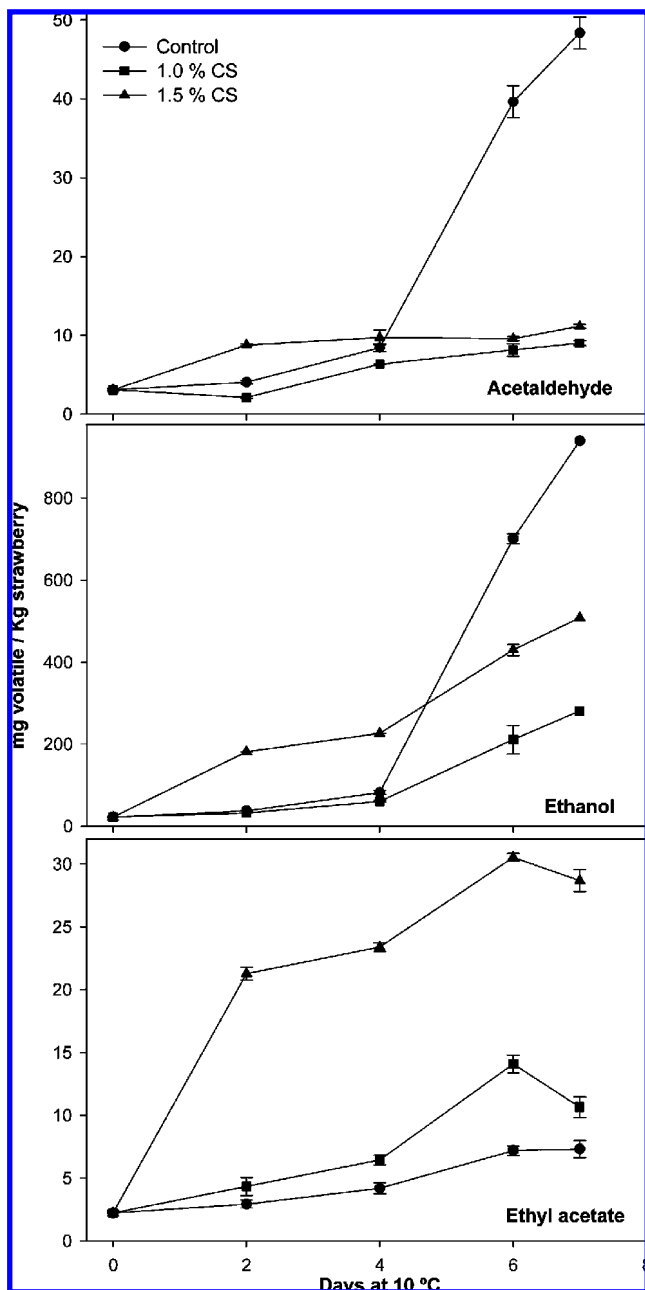
Figure 1. Evolution of acetaldehyde, ethanol, and ethyl acetate concentrations (mg of volatile/kg of strawberry) in chitosan-coated and uncoated strawberries stored for 7 days at 10 °C.

of controlled atmosphere conditions with CO_2 concentrations up to 24%, O_2 concentrations down to 1%, or a combination of 10% CO_2 and 2% O_2, Larsen and Watkins (5) reported an increase in the levels of ethanol and ethyl acetate but not acetaldehyde and related this increment with the development of off-flavors. Ke et al. (10) correlated the accumulation of ethanol and, to lesser extents, ethyl acetate and acetaldehyde with the development of off-flavors in strawberries kept in 0.25 or 0% O_2. Exposing strawberries to 0.25% O_2, 21% O_2 + 50% CO_2, or 0.25% O_2 + 50% CO_2 at 5 °C for 1−7 days, Ke et al. (14) found a considerable increase in the concentrations of acetaldehyde, ethanol, and ethyl acetate over those of air-control fruit. A 0.25% O_2 atmosphere caused the greatest increase in ethyl acetetate.

Figure 1 shows the evolution of the concentrations of acetaldehyde, ethanol, and ethyl acetate in control and coated strawberries. For each sample the concentration of the metabo-

lites increased during storage, albeit with different evolution profiles. The levels of acetaldehyde and ethanol increased progressively in uncoated fruit, undergoing a sharp rise after the fourth day of storage. Coated strawberries showed a progressive and significantly slower buildup of acetaldehyde and ethanol compared to uncoated fruit. However, prior to the fourth day of storage, the levels of acetaldehyde and ethanol were higher in 1.5% chitosan-coated strawberries compared to the control. Strawberries coated with 1% chitosan presented lower levels of acetaldehyde and similar levels of ethanol compared to control fruit. At the end of the storage period, the concentrations of both volatiles were much lower in coated fruit. This reduced accumulation of acetaldehyde and ethanol in coated fruit may be a positive indicator of prolonged shelf life. Accumulation of acetaldehyde and ethanol in air-stored fruit is associated with overmaturation and microbial infection (19, 20). It is noteworthy that this correlates well with previous studies carried out on the effect of chitosan coatings on the quality of strawberries stored at 10 °C (6). In that study, it was found that untreated fruit experienced an acute decrease in the *L*, hue, and chroma color parameters, as well as a dramatic lost of firmness on the sixth day of storage. Ten percent of the untreated fruits also showed visual fungal growth and dehydration. Chitosan-coated fruit did not show fungal decay or loss of color at this stage of storage.

By comparison of the effect of the chitosan coating concentration on the evolution of fermentative metabolites, strawberries treated with a higher concentration of chitosan presented a greater content of fermentative metabolites. This behavior is indicative of lower O_2 and higher CO_2 levels reached within strawberries coated with a greater concentration of chitosan.

Ethyl acetate levels increased more quickly in coated strawberries relative to uncoated controls, and this effect was enhanced at higher chitosan concentrations. Accumulation of ethyl acetate is likely to occur under anaerobic conditions created by modified atmospheres, controlled atmospheres, or coatings more than in overripe fruit (12). Thus, it is expected that higher levels of ethyl acetate would be found in coated fruit, where partially anaerobic conditions can be reached. The values of ethyl acetate observed for all of the treatments (from 10 to 29 mg/kg of strawberry) are within the limits (63 μL/L) reported as being acceptable by Ke et al. (10) and not related to off-flavors. The concentrations of acetaldehyde, ethanol, and ethyl acetate observed in samples coated with 1% chitosan are within the ranges reported by Pesis et al. (21), which resulted in an enhancement in fruit flavor. Thus, the treatment of strawberries with 1% chitosan coating could be linked to an improvement of the strawberry aroma profile.

In summary, until the fourth day of storage, strawberries coated with 1% chitosan accumulated lower amounts of fermentative metabolites than those treated with 1.5% chitosan. There were no significant differences in ethanol concentration between 1% chitosan-coated fruit and control fruit, whereas the levels of acetaldehyde and ethyl acetate in 1% chitosan-coated berries were close to those found in control fruit. The decay of untreated fruit would explain the sharp rise in acetaldehyde and ethanol levels after the fourth day of storage and the greater accumulation of these metabolites compared to coated berries.

Alcohols. Methanol and hexanol are important for strawberry flavor development (22). The evolution of these alcohols along with other key alcohols such as *cis*-3-hexen-1-ol, β-citronellol, and benzyl alcohol was studied in uncoated and chitosan-coated strawberries. As illustrated in **Table 1**, methanol, 1-hexanol, and *cis*-3-hexen-1-ol concentrations

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Table 1. Effect of Chitosan Coatings on the Concentration (Milligrams per Kilogram) of Selected Volatile Compounds in Strawberries Stored at 10 °C for 1 Week[a]

compound	treatment	day 0	day 2	day 4	day 6	day 7
methanol	control		20.7 ± 0.2a	20.9 ± 0.4a	22.6 ± 0.7a	23.1 ± 0.4a
	1% CS	19.7 ± 0.3	19.5 ± 0.3b	19.4 ± 0.2b	19.9 ± 0.3b	20.3 ± 0.4b
	1.5% CS		18.8 ± 0.2c	19.5 ± 0.2b	20.1 ± 0.7b	20.5 ± 0.3b
1-hexanol	control		0.44 ± 0.02a	0.53 ± 0.02a	0.74 ± 0.03a	0.66 ± 0.06a
	1% CS	0.41 ± 0.09	0.45 ± 0.03a	0.51 ± 0.03a	0.40 ± 0.03b	0.31 ± 0.05b
	1.5% CS		0.32 ± 0.03b	0.36 ± 0.07b	0.30 ± 0.04c	0.28 ± 0.04b
β-citronellol	control		0.13 ± 0.01a	0.18 ± 0.02a	0.17 ± 0.03a	0.20 ± 0.02a
	1% CS	0.038 ± 0.005	0.055 ± 0.007b	0.038 ± 0.017b	0.062 ± 0.006b	0.092 ± 0.002b
	1.5% CS		0.049 ± 0.005b	0.084 ± 0.007c	0.045 ± 0.006c	0.092 ± 0.008b
cis-3-hexen-1-ol	control		2.47 ± 0.02a	2.92 ± 0.17a	2.39 ± 0.05a	2.47 ± 0.04a
	1% CS	1.93 ± 0.01	1.63 ± 0.03b	1.53 ± 0.06b	2.16 ± 0.25b	1.85 ± 0.08b
	1.5% CS		1.58 ± 0.03b	1.73 ± 0.07c	1.44 ± 0.09c	1.80 ± 0.08b
benzyl alcohol	control		0.018 ± 0.001a	0.018 ± 0.001a	0.022 ± 0.001a	0.037 ± 0.005a
	1% CS	0.012 ± 0.001	0.013 ± 0.001b	0.012 ± 0.001b	0.015 ± 0.001b	0.020 ± 0.001b
	1.5% CS		0.011 ± 0.001c	0.012 ± 0.001b	0.012 ± 0.001c	0.020 ± 0.001b
acetic acid	control		1.96 ± 0.12a	1.95 ± 0.23a	3.60 ± 0.79a	6.40 ± 0.90a
	1% CS	1.90 ± 0.15	1.47 ± 0.12b	0.82 ± 0.13b	1.78 ± 0.29b	0.39 ± 0.30b
	1.5% CS		1.69 ± 0.12b	1.99 ± 0.13a	3.80 ± 0.39a	4.46 ± 0.11c
hexanoic acid	control		7.17 ± 0.99a	7.83 ± 0.46a	7.05 ± 0.33a	8.11 ± 0.25a
	1% CS	0.70 ± 0.05	1.00 ± 0.07b	0.93 ± 0.06b	1.00 ± 0.02b	2.84 ± 0.21b
	1.5% CS		1.00 ± 0.07b	1.08 ± 0.07b	0.70 ± 0.04b	2.74 ± 0.21b
hexanal	control		16.81 ± 0.72a	7.47 ± 0.58a	1.85 ± 0.81a	0.65 ± 0.19a
	1% CS	14.90 ± 1.05	15.44 ± 0.81a	9.81 ± 0.51b	6.12 ± 0.51b	2.99 ± 0.53b
	1.5% CS		9.53 ± 0.83b	8.45 ± 0.73a	2.59 ± 0.73a	3.59 ± 0.83b
methyl butanoate	control		0.91 ± 0.02a	1.03 ± 0.01a	1.17 ± 0.018a	0.93 ± 0.01a
	1% CS	0.51 ± 0.02	0.85 ± 0.05a	0.56 ± 0.03b	0.60 ± 0.02b	0.22 ± 0.01b
	1.5% CS		0.14 ± 0.05b	0.17 ± 0.03c	0.18 ± 0.01c	0.024 ± 0.09c
ethyl butanoate	control		2.80 ± 0.11a	3.78 ± 0.30a	3.33 ± 0.30a	2.29 ± 0.06a
	1% CS	0.41 ± 0.03	4.11 ± 0.30b	3.46 ± 0.33a	5.35 ± 0.12b	3.97 ± 0.13b
	1.5% CS		4.62 ± 0.20b	5.70 ± 0.34b	4.52 ± 0.07c	3.87 ± 0.06b
ethyl hexanoate	control		5.93 ± 0.92a	10.25 ± 1.20a	6.23 ± 1.03a	4.51 ± 0.59a
	1% CS	0.12 ± 0.02	3.71 ± 0.95b	3.11 ± 0.85b	6.55 ± 1.03a	7.80 ± 0.99b
	1.5% CS		8.02 ± 0.90c	6.13 ± 0.82c	4.98 ± 1.02a	5.59 ± 0.42c
hexyl acetate	control		3.51 ± 0.31a	4.35 ± 0.11a	4.69 ± 0.40a	4.89 ± 0.06a
	1% CS	2.89 ± 0.22	2.99 ± 0.20b	3.01 ± 0.36b	3.70 ± 0.31b	4.24 ± 0.07b
	1.5% CS		3.03 ± 0.31b	3.06 ± 0.14b	2.93 ± 0.04c	3.17 ± 0.37c
octyl acetate	control		2.48 ± 0.07a	2.69 ± 0.02a	2.70 ± 0.02a	2.72 ± 0.07a
	1% CS	2.21 ± 0.06	2.27 ± 0.07b	2.25 ± 0.07b	2.37 ± 0.05b	2.54 ± 0.05b
	1.5% CS		2.21 ± 0.05b	2.32 ± 0.05b	2.34 ± 0.03b	2.46 ± 0.14b
2-nonanone	control		0.0179 ± 0.0009a	0.0176 ± 0.0005a	0.0141 ± 0.0005a	0.0152 ± 0.0002a
	1% CS	0.0165 ± 0.0012	0.0143 ± 0.0009b	0.0123 ± 0.0010b	0.0176 ± 0.0006b	0.0204 ± 0.0006b
	1.5% CS		0.0130 ± 0.0005b	0.0123 ± 0.0005b	0.0152 ± 0.0002c	0.0192 ± 0.0005c
2-heptanone	control		0.0109 ± 0.0003a	0.0010 ± 0.0003a	0.0097 ± 0.0001a	0.0091 ± 0.0003a
	1% CS	0.0113 ± 0.0005	0.0099 ± 0.0002b	0.0094 ± 0.0002b	0.0089 ± 0.0003b	0.0082 ± 0.0004b
	1.5% CS		0.0082 ± 0.0003c	0.0082 ± 0.0006c	0.0074 ± 0.0003c	0.0075 ± 0.0003c
Furaneol	control		3.63 ± 0.07a	1.50 ± 0.18a	0.89 ± 0.03a	0.68 ± 0.03a
	1% CS	2.15 ± 0.12	3.91 ± 0.09b	3.18 ± 0.19b	2.72 ± 0.11b	1.42 ± 0.22b
	1.5% CS		3.07 ± 0.05c	1.38 ± 0.05a	1.16 ± 0.21a	0.79 ± 0.13a

[a] For each volatile, means (concentration ± standard deviation, $n = 3$) with different letters within columns differ significantly ($p \leq 0.05$).

increased with storage time in uncoated fruit, whereas they remained almost the same in chitosan-treated fruit throughout storage. Methanol accumulation has been reported to occur during the postharvest aging of strawberries; thus, the constant level of this volatile during storage could be related to the effect of chitosan delaying senescence. Fruit over-

ripeness could explain the higher concentration of 1-hexanol found in uncoated strawberries with respect to coated ones after the fourth day of storage. Membrane disruption associated with fruit senescence leads to the release of fatty acids, increasing lipid peroxidation and the biosynthesis of six-atom carbonyls and alcohols (*23*).

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With regard to the aroma compounds β-citronellol and benzyl alcohol, concentrations increased with storage, reaching a maximum after 7 days, but this increase was slower ($p \leq 0.05$) in chitosan-treated fruit compared to uncoated controls. Because these compounds have been related to floral and fruity notes, their lower concentration in coated fruit could induce delayed accumulation of the aroma note. The observed slow increase in alcohol concentrations in coated strawberries could be nondetrimental to the overall aroma profile of those fruits because it is thought that these alcohols, even if numerous, normally contribute little to strawberry aroma. Because the accumulation of alcohols is primarily associated with anaerobic atmospheres or with CO_2-rich atmospheres, the results presented for alcohols are in agreement with those for fermentative metabolites in the sense that anaerobic conditions have seemingly not developed in coated strawberries.

Acids and Aldehydes. Acid components of strawberry aroma such as acetic acid and hexanoic acid were analyzed in uncoated and coated fruits over 7 days of storage at 10 °C (**Table 1**). Large differences ($p \leq 0.05$) in the levels of both volatiles after several days of storage were found between untreated and treated fruits. At the end of storage, undesirable increases in hexanoic and acetic acid contents were observed in uncoated strawberries. For control fruit, the hexanoic acid content was higher by a factor of about 8 after 2 days, whereas all coated fruits maintained essentially the same level for 6 days. Acetic acid concentration began to increase in uncoated and 1.5% chitosan-coated strawberries after 4 days of storage, and this increase became greater for uncoated fruit at the end of storage. However, the concentration of acetic acid did not increase in 1% chitosan-coated strawberries throughout storage.

Hexanoic acid along with 2-methylpropanoic, butanoic, and octanoic acids are predominant in strawberries and are precursors for the formation of esters (*24*). Hexanoic acid is associated with unpleasant smells of sweet and rancid. Thus, the reduced buildup of hexanoic acid could be a sign of enhanced strawberry aroma conservation during storage. In **Table 1** it can be seen that acetic acid and hexanoic acid show different responses to variations in the chitosan concentration. Hexanoic acid was barely affected by the difference in the chitosan concentration, whereas the acetic acid concentration showed pronounced differences. Acetic acid production could be affected more by the internal levels of CO_2 and O_2 reached by means of the amount of coating applied. Larsen and Watkins (*12*) reported an increase in the acetic acid level in air-stored strawberries and a greater increase in air + 20 kPa of CO_2. The same authors found that the level of hexanoic acid increased in air and decreased in fruit stored in air + 20 kPa of CO_2.

The six-carbon aldehydes present in strawberry aroma such as hexanal are produced naturally via the lipoxygenase-lyase oxidation of linoleic and linolenic acids and then metabolized to alcohols and esters. As shown in **Table 1**, the hexanal concentration decreased during storage by up to 93%. This could be related to the conversion of hexanal to its corresponding alcohol, 1-hexanol, and ester, hexyl acetate, as reported in the literature (*25*). The most rapid decrease in hexanal levels was observed for control fruit, but the differences between coated and uncoated berries were rather minor. By comparison of the different coatings it can be observed that hexanal reduction was more pronounced for the higher chitosan concentration except for the last day of storage.

Esters. Esters are both quantitatively and qualitatively the most abundant class of volatile compounds in strawberry fruit. They comprise 25−90% of the total volatiles in fresh ripe fruit

depending on the cultivar (*26*). More than a hundred different esters have been identified in strawberry aroma (*27*), providing fruity and floral notes. Methyl and butyl esters of butanoic and hexanoic acid are quantitatively the major esters found in mature strawberries (*1, 3, 24, 28*). The formation of esters occurs only at mature stages due to an increase in strawberry alcohol acyltransferase (AAT) activity through maturation (*29*). This enzyme catalyzes the transfer of an acyl moiety of an acyl CoA onto a corresponding alcohol, giving rise to the formation of an ester. The content of esters contributing to the aroma of ripe strawberry has been reported to increase during fruit storage (*30, 31*). However, the ester profile of strawberries changes during postharvest life depending on cultivar variety, storage period, and the storage environment including atmosphere, temperature, and light (*26*). **Table 1** shows the initial concentration and evolution of some of the main esters present in coated and uncoated strawberries. As can be seen, the concentrations of most esters increased ($p \leq 0.05$); therefore, fruity and floral notes were enhanced during storage. After 7 days, hexyl acetate, ethyl butanoate, and ethyl hexanoate exhibited the largest increases in concentration compared to day 0.

Comparing treatments, the evolution of ethyl esters was similar in coated and uncoated fruits and the concentrations of ethyl butanoate and ethyl hexanoate increased during storage. Nonetheless, at the end of storage, strawberries coated with chitosan showed higher levels ($p \leq 0.05$) of ethyl esters compared to control. Acetate esters also increased during storage, but contrary to the results found for ethyl esters, hexyl acetate and octyl acetate concentrations were lower in coated strawberries ($p \leq 0.05$). The initial concentration of methyl butanoate increased during storage in control fruit and decreased in fruit coated with 1.5% chitosan. Fruit coated with 1% chitosan kept the initial levels of methyl butanoate until the sixth day of storage. Comparing coating treatment, the higher chitosan concentration gave rise to lower levels of hexyl acetate and methyl butanoate over the storage period. Thus, the concentration of chitosan had different effects on different esters, but no trend was observed. Flavor differences could thus be expected for coated berries. However, sensorial test assessment should be carried out to correlate variation detected by analytical instruments and human perception.

As mentioned above, AAT plays a crucial role in ester biosynthesis. Several studies have shown that the pattern of volatile esters in fruit depends on both AAT substrate specificity and the availability of acyl CoA molecules and alcohols as substrates. Perez et al. (*22*) examined the affinity of the enzyme for several alcohols using acetyl-CoA and found maximum activity with hexanol. The authors established a clear correlation between AAT substrate preference and the distribution of different types of volatile esters in strawberry aroma. The higher levels of hexanol and methanol found in uncoated samples could explain the greater production of hexyl acetate and methyl butanoate. Accordingly, Hamilton-Kemp et al. (*25*) demonstrated that strawberry fruit readily converted exogenous six-carbon alcohols such as 1-hexanol and *cis*-3-hexen-1-ol, both detected in this study, to their corresponding volatile esters.

Ester biosynthesis is greatly affected by low-O_2 and/or high-CO_2 atmospheres. A large increase in the content of ethyl esters and a decrease in methyl esters and butyl and hexyl acetates have been observed for strawberries kept in air + 20 kPa of CO_2 (*13, 15*). The accumulation of ethanol increases the consumption of acetyl CoA and other carboxylic groups for the biosynthesis of ethyl acetate and other ethyl esters, therefore reducing the production of other esters (*15*). Ke et al. (*14*)

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observed an increase in the production of ethyl acetate and ethyl butyrate as well as a decrease in the production of isopropyl, butyl, and hexyl acetate in strawberries exposed to several controlled atmospheres. Along with the accumulation of ethanol, the authors found a slight decrease in AAT activity. Perez et al. (*29*) reported a slight increase in AAT activity in strawberries packaged in a passive modified atmosphere after 9 days at 17 °C (>50% CO_2, <1% O_2) with an ester pattern in which methyl and ethyl acetates were the major compounds accompanied by traces of other esters. In the present study, the level of ethyl butanoate in coated strawberries was around 70% higher than in control fruit, and ethyl hexanoate increased by 73 and 24% for 1 and 1.5% chitosan-coated fruit, respectively, after 7 days of storage. Although the levels of ester acetates in coated strawberries were not found to diminish, their rate of production was lower compared to uncoated berries. These results suggest that the esters observed in chitosan-coated strawberries are related to a more favorable pattern of internal gas composition with a higher content of O_2 and a lower content of CO_2 than reported in the above-mentioned studies.

Jetti et al. (*32*) evaluated the odor activity values of aroma compounds of some strawberry cultivars and correlated them with sensory descriptive analysis. The Camarosa cultivar was found to have lower fruity notes, which correlated well with lower total active odor values for esters. However, the sensorial perception of green notes did not correlate with the low odor values obtained for alcohols and aldehydes, which are responsible for green aroma notes. The authors concluded that the large amounts of green notes were probably due to the lack of the fruity notes. On the basis of the application of aroma extract dilution analysis on fresh strawberry juice, Schieberle and Hoffmann (*1*) selected 15 odor active compounds having high flavor dilution factors. Among the esters selected, methyl butanoate and ethyl butanoate were found to be potent odorants in fresh strawberry juice having the highest odor active values. This was corroborated with sensory experiments on model mixtures. Evaluating the aroma composition of some strawberry cultivars, Larsen et al. (*33*) concluded that among the esters found in strawberries, ethyl butanoate and ethyl hexanoate made very important contributions to the sensory impression of strawberry because these compounds presented the highest active odor values (concentration/threshold). Whereas ethyl hexanoate was found to be a general strawberry aroma compound, ethyl butanoate was found to be cultivar-specific. In summary, (1) the increases in ethyl hexanoate and ethyl butanoate and (2) the absence of variations in the initial levels of other important esters along with (3) a decrease in volatiles contributing to green notes may enhance the flavor quality of strawberries coated with chitosan.

Furans and Ketones. 2,5-Dimethyl-4-hydroxy-2*H*-furan-3-one (Furaneol) and its methoxy derivative (mesifurane) are important contributors to strawberry aroma. Although quantitatively they are minor constituents of the flavor of the fruit, they are highly influential on overall flavor because they have such low threshold values (*27*). By application of aroma extract dilution analysis to fresh strawberry juice, Schieberle and Hoffmann (*1*) found Furaneol and mesifurane to have high flavor dilution factors, with Furaneol having greater values than mesifurane. The authors reported Furaneol as a potent odorant with the greatest odor active value of 15 volatile compounds determined in fresh strawberry juice. Furaneol, along with (*Z*)-3-hexenal and methyl butanoate, was also found to have a great character impact in model mixtures of strawberry odors in a synthetic juice matrix.

Table 1 shows the evolution of the concentration of Furaneol in uncoated and coated strawberries. After several days of storage, the Furaneol concentration showed a slight decrease for all treatments. The rate of decrease differed depending on the chitosan treatment used. Strawberries coated with 1% chitosan showed reduced Furaneol content of about 31%, whereas a 64% reduction was observed for the 1.5% coating and the control. The decrease in Furaneol content during strawberry storage could be related to this conversion into the derivatives mesifurane and the glucoside Furaneol. The rapid conversion of Furaneol to its derivatives has been reported in ripe strawberries (*34, 35*). Furaneol has better organoleptic properties and a lower odor threshold than mesifurane (*2*). Furaneol has a sweet and burnt aroma but is not strawberry-like except when mixed with ethyl butanoate (*2*). Therefore, the combination of a greater retention of Furaneol during storage together with the observed increment in ethyl butanoate levels could indicate an enhancement in the aroma of strawberries coated with 1% chitosan.

Ketones of strawberry aroma such as 2-heptanone and 2-nonanone were markedly affected by chitosan concentration and storage time (**Table 1**). During storage at 10 °C, coated strawberries showed greater losses of 2-hepatone compared to the control (8 and 15% in berries coated with 1 and 1.5% chitosan, respectively). **Table 1** also shows significantly different evolutions of the 2-nonanone levels between uncoated and coated strawberries. After 7 days of storage at 10 °C, coated berries showed higher levels of 2-nonanone compared to the control, with the highest concentration being observed for the strawberries coated with 1% chitosan.

On the basis of the experimental results presented in this work, chitosan coating technology is able to reduce the accumulation of acetaldehyde and ethanol, increase the occurrence of ethyl esters (fruity and floral notes), and slow the buildup of alcohols and volatile acids in strawberries stored at 10 °C for 1 week. Sensorial analysis needs to be carried out to evaluate the effect of these changes on human perception.

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