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Chitosan: Antimicrobial activity and potential applications for preserving minimally processed strawberries

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ABSTRACT

In this research the possible use of chitosan coating on fresh-cut strawberries was investigated. Manually sliced strawberries were treated with a solution of 1% chitosan, packaged in modified atmosphere with high (80%) and low (5%) percentage of oxygen and then stored at 4, 8, 12 and 15 °C. Changes in microbiological quality were measured and the shelf life of the samples, as stability time, was kinetically modelled in order to check the effects of storage temperature on the most relevant microbial indices for product quality.

A chitosan coating inhibited the growth of microorganisms and affected significantly and positively the stability time of the products, above all when the samples were packaged in modified atmosphere (with low and high percentage of oxygen). Besides, the presence of high percentage of oxygen, combined with chitosan coating, seemed to affect positively the colour.

The data revealed that applying a chitosan coating prolonged effectively the quality and extended the shelf life of fresh-cut strawberries.

1. Introduction

Strawberries (*Fragaria* × *ananassa* Duch.), that are one of the most popular fruits worldwide, are rich in nutrients (amino acids, vitamins and anthocyanins) but also highly perishable, being susceptible to mechanical injury, desiccation, decay and physiological disorders during storage. Consequently, they have short ripening and senescent periods that make marketing a challenge (Garcia et al., 1998a, b). Elevated CO_2 levels and low temperatures are effective in reducing the incidence of decay, even if prolonged exposure of berries to high CO_2 concentrations can cause off-flavour development (Ke et al., 1994). The shelf life of fresh strawberries at cold temperature is usually less than 5 days. This storage time is reduced when the product is minimally processed.

A fresh-cut product is defined by the International Fresh-cut Produce Association as fruit or vegetables that have been trimmed and/or peeled and/or cut into 100% usable product, that is bagged or pre-packaged to offer consumers high nutrition, convenience and flavour while still maintaining freshness (www.fresh-cut.org). Improvements in shelf-life can be achieved by using good quality raw products, special care during processing and along the trade chain, control of temperature and relative humidity, and use of modified atmosphere packaging (King and Bolin, 1989; Nguyen-The and Carlin, 1994).

Respiring products, like fruits, processed vegetables and mixed salads, generate equilibrium gas conditions inside the package, that are very low in O_2 (2–3%) and moderately high in CO_2 (Amanatidou et al., 1999). These conditions reduce the proliferation of spoilage aerobic microorganisms (Moleyar and Narasimham, 1994). The antimicrobial effect of CO_2 is well documented; moreover recent experimental trials have indicated that high O_2 percentage (70–90%) may be advantageous for fresh-cut quality (Day, 1996). The use of high levels of O_2 for packaging respiring products, however, is still in its infancy and needs to be supported by research. Little literature is available; however the toxic effect of O_2 on microbial growth due to the formation of superoxide radicals and their effect on cell metabolism have been already explained (Gregory and Fridovich, 1973).

Recently, application of edible coatings is promising to improve the quality and extend shelf life of lightly processed produce (Li and Barth, 1998), because they act as barriers to water loss and gas exchange, creating a micro-modified atmosphere around products, and can serve as carriers for other GRAS compounds (Baldwin et al., 1995). Chitosan, might be an ideal preservative coating for fresh fruits because of its film-forming and biochemical properties (El Ghaouth et al., 1992) and its use in food is particularly promising because of its "biocompatibility", non-toxicity and antimicrobial action.

Chitosan has been used to maintain the quality of post-harvest fruits and vegetables such as citrus (Chien et al., 2007), peach, pear and kiwifruit (Du et al., 1997), strawberries (El Ghaouth et al., 1991), tomatoes (El Ghaouth et al., 1992), apples (Ippolito et al.,

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2000), longan fruit (Jiang and Li, 2001), litchi fruit (Zhang and Quantick, 1997). Besides the effects of chitosan coating on the storage life of fresh-cut fruits such as Chinese water chestnut (Pen and Jiang, 2003), litchi (Dong et al., 2004) strawberries (Devlieghere et al., 2004) and mango (Chien et al., 2007) was investigated.

In this study, an evaluation of the potential use of chitosan as a food preservative for fresh-cut strawberries (*Fragaria* × *ananassa* Duch.) in relation to package atmosphere, with low and high percentage of O_2, and storage temperature was investigated.

2. Methods

2.1. Fresh cut strawberries preparation

Strawberries were purchased from retail stores. After the calyxes had been removed, the berries were washed with tap water and half sliced along the two distal parts. Only sliced fruit with no external injury were selected.

Strawberries were dipped for 5 min in a chitosan solution, drained and put to dry. Control samples were dipped in distilled water.

Chitosan solution (1%, w/v) was prepared by using low molecular weight chitosan (50–160 kDa) with a deacetylation degree of 75–85% (Sigma-Aldrich, Milan, Italy), in 1% citric acid (J.T. Baker, Milan).

The slices were packaged in high (HP) and low permeability (LP) bags (Tecnovac, San Paolo D'Argon, Bergamo, Italy)] by means of S100-Tecnovac equipment. The bags were 170 × 250 mm long with properties specified by the manufacturer as follows: bags at low permeability were characterized by CO_2 and O_2 permeability of 3.26×10^{-19} and 9.23×10^{-19} mol m m^{-2} s^{-1} Pa^{-1} respectively and water vapour transmission rate of 1.62×10^{-10} kg m^{-2} s^{-1}; bags at high permeability were characterized by CO_2 and O_2 permeability of 3.36×10^{-8} and 7.03×10^{-19} cm^3 (STP) cm cm^{-2} s^{-1} atm^{-1}. The samples were packaged in air (control atmosphere, CA) in high permeability bags; in air, modified atmosphere 1 (MA1: 65% N_2, 30% CO_2, 5% O_2) and modified atmosphere 2 (MA2: 80% O_2, 20% CO_2) in bags at low permeability.

The samples were stored at 4, 8, 12 and 15 °C. Microbiological analyses, determination of pH, soluble solids and colour and the evaluation of the amounts of O_2 and CO_2 in the bags were performed within the storage.

2.2. Fruit sampling and microbiological analyses

For microbiological analysis, 10 g of strawberry slices were diluted with 90 ml of sterile saline solution (9 g/l NaCl) in a Stomacher bag (Seward, London, England) and blended for 1 min in a Stomacher Lab Blender 400 (Seward). Serial dilutions of fruit homogenates were plated on selective media and incubated under appropriate conditions.

The media and the conditions used were as follows: Plate Count Agar (PCA) incubated at 5 °C for a week or at 32 °C for 48 h for psychrotrophic and mesophilic bacteria, respectively; Violet Red Bile Glucose Agar (VRBGA) incubated at 37 °C for 18–24 h for *Enterobacteriaceae*; MRS agar+cycloheximide (0.17 g/l) (Sigma) incubated at 30 °C for 4 days under anaerobiosis for lactic acid bacteria; Sabouraud Dextrose Agar+chloramphenicol (0.1 g/l) (Carlo Erba, Milan) incubated at 28 °C for 2 days for yeasts; Malt Extract Agar (MEA) incubated at 25 °C for 4 days for moulds. All media used were from Oxoid (Milan, Italy).

Since the media used were not highly selective, the results for plate counts were confirmed by microscopic observations.

Microbiological data were expressed as the average of at least two replicates. The variability coefficients, expressed as the percentage ratio between the standard deviation and the mean value, were less than 7%.

The cell load data of each microbial group, collected during the storage of the products, were modelled according to Gompertz equation modified by Zwietering et al. (1990):

$$y = k + A\exp\{-\exp[(\mu_{max}\, e/A)(\lambda - t) + 1]\}$$

where y is the log [CFU/g], k is the initial level of the dependent variable to be modelled, A is the maximum increase of bacterial load attained at the stationary phase, μ_{max} is the maximal growth rate (Δ log [CFU/g]/day), λ is the lag time (days) and t is the time.

The experimental data were modelled through the Non-Linear Regression Procedure of the statistic package Statistica for Windows (Statsoft, Tulsa, OK).

The measurement of pH was performed with a Crison pH metre model micro pH 2001 (Crison, Barcelona, Spain) on the strawberry samples diluted $\frac{1}{10}$ in a saline solution and blended for 1 min in a Stomacher Lab Blender 400 (Seward).

2.3. Shelf-life modelling

The microbiological shelf-life was calculated as stability time, that can be assessed at the maximum acceleration of microbial growth (dN^2/dt^2) estimated with the Gompertz function.

The underlying principle implied that the microbial spoilage had to show a rate of the same order as at the shelf-life zero time. This condition was no longer met when microbial growth attained its maximal acceleration, because the system underwent very fast changes with the loss of acceptability and safety (Riva et al., 2001).

This principle seems more reliable than the current practice that defines food stability according to the ratio between attained and starting microbial population levels.

The relationship between acceptability times (At) for bacteria and temperature was represented by a linear equation:

$$\mathrm{At} = a + bT$$

where a is theoretical shelf-life at 0 °C, T is temperature (°C) and b (the slope) represents the increase of degradative reaction rates for each 1 °C temperature increase (Singh, 1994). This parameter multiplied by a factor 10 is known as Q_{10} value.

2.4. Phenotypic identification of microorganisms

2.4.1. Psychrotrophic bacteria

For the identification, three colonies of each different bacterial morphological type were selected from the primary cultures and kept on Plate Count Agar (Oxoid) at 4 °C until they were identified. All bacteria strains were grouped on the basis of staining reaction, catalase test, oxidative-fermentative metabolism of glucose, motility reaction, cell shape and spore formation by heating cultures at 80 °C for 10 min and successive plating on PCA according to Collins et al. (1989). The isolates were identified at the species level, using the appropriate API identification system (BioMerieux, Marcy l'Etoile, France).

2.4.2. Lactic acid bacteria

For the identification, presumptive mesophilic lactic acid bacteria isolates were characterized by physiological and biochemical assays (Devriese and Pot, 1995; Teuber, 1995; Hammes and Vogel, 1995) and API 50 CHL systems (API system, BioMerieux, Marcy l'Etoile, France).

2.4.3. Yeasts

For the identification three colonies of each different yeast morphological type were selected from the primary cultures and kept on Sabouraud Dextrose Agar (Oxoid) at 4 °C until they were identified. The isolates were characterised according to the method of Van der Walt and Yarrow (1994) and by using the API ATB ID32C system (BioMerieux). Identification was carried out by comparing the test results with the tables of Kurtzman and Fell (1998).

2.5. Colour measurements

Colour analysis was made on the inner and outer section of sliced strawberries. The changes in colour during the storage at 4, 8, 12 and 15 °C were monitored by colorimetric measurements using a Tristimulus Colorimeter Chromameter-2 Reflectance (Minolta, Osaka, Japan) equipped with a CR-300 measuring head. The instrument was standardized against a white tile before each determination. The colour of sliced strawberry was determined as L', a' and b' values (CIELAB); in this work the changes in colour were expressed as a'/b' (Porretta, 1991). Data were the average of at least five repetitions. The variability coefficients, expressed as the percentage ratio between the standard deviation and the mean value, were $<5\%$.

2.6. Soluble solids determination

Determination of sugar content expressed as Brix degrees, were made on strawberry juice, by Abbe refractometer. The obtained data were submitted to one-way ANOVA and Duncan's test ($p<0.05$) through the software Statistica for Windows.

2.7. Gas evaluation

The amounts of the O_2 and CO_2 in low permeability bags were evaluated at selected time intervals within the storage time through a head space gas analyser Checkmate II (Pbi Dansensor, Ringsted, Denmark). Analyses were performed in duplicate and the amounts of gas were expressed as a v/v percentage.

3. Results and discussion

3.1. Influence of atmosphere composition and storage temperature on microbial growth

For a better understanding of the effect of the atmosphere composition and storage temperature on microbiological quality of fresh-cut strawberries (with and without chitosan coating), three different atmospheres (air, CA; modified atmosphere 1, MA1—65% N_2, 30% CO_2, 5% O_2; modified atmosphere 2, MA2—80% O_2, 20% CO_2) and four temperatures (4, 8, 12 and 15 °C) were used. Moreover, as regard to CA two different bags were used: bags at high (HP) and low permeability (LP).

The microbiological shelf life is usually calculated as the time necessary to attain a total bacterial count of 5×10^7 CFU/g, as recommended by French regulations for fresh-cut vegetables (Ministere de l'Economie des Finances et du Budget, 1998). In our experimental conditions, the low pH (c.a. 3.5) of strawberries did not allow the reaching of this cell load; therefore the shelf life was calculated as stability time.

The obtained data were modelled through the modified Gompertz equation; the three parameters defined by the model (maximum increase of cell load attained at the stationary phase, A; maximal growth rate, μ_{max}; and lag phase, λ), the regression coefficient and stability time for mesophilic bacteria are shown in Table 1.

Under refrigeration, the antimicrobial activity of chitosan seemed to influence significantly the growth of mesophilic bacteria in CA, if combined with the bags at low permeability, as one could infer by the lower A values (2.66 and 3.89 log CFU/g in the coated strawberries and in the control samples, respectively, at 4 °C) as well as by the decrease of growth rate and the prolongation of lag phase; on the other hand, with increasing of the storage temperature, the differences between the chitosan treated fruits and the controls were slight and less significant.

The use of bags at high permeability resulted in a faster decay of the samples, both in chitosan coated and uncoated strawberries, as a visible mould growth was recovered after 12, 7 and 5 days at 8, 12 and 15 °C, respectively. This result agreed with the data of Soliva-Fortuny et al. (2004), who reported that the inhibition of mould and yeast growth was not so marked in fresh-cut apples packaged with plastic of higher permeability.

Due to mould growth on strawberries packed in high permeability bags, shelf life evaluation, along with microflora identification and chemico-physical evaluations will be discussed in the following sections only for strawberries packaged in low permeability bags.

A second hurdle to inhibit microbial growth was the use of MAP. In fact, it is really interesting to underline that at 4 and 8 °C the modified atmospheres (MA1 and MA2), were able to control mesophilic bacteria, whose cell load was about 3 log CFU/g in the controls; the use of chitosan caused a further inhibition of bacteria, as no growth was observed within the entire storage time. Besides, at 12 and 15 °C the use of chitosan coating prolonged the lag phase and slowed the growth rate.

Many author have demonstrated the advantages of conventional MAP using 3–5 kPa of O_2 and 5–10 kPa of CO_2 (balanced with N_2) to reduce deterioration of fresh processed vegetables and proliferation of aerobic spoilage microorganisms (Kader, 1986; Gorris and Peppelenbos, 1992; Nguyen-the and Carlin, 1994; Ahvenianen, 1996; Artés and Martinez, 1996). Nevertheless it is known that the effect of conventional MAP on aerobic mesophilic microflora is variable. Actually, atmospheres with O_2 levels higher than 70 kPa, have recently been suggested as an innovation to modified atmosphere packaging (MAP) for fresh processed vegetables to maintain sensory quality and safety (Allende et al., 2002).

The stability time was calculated through the Gompertz parameters and this value is showed in Table 1. Stability time values were affected both by dipping in chitosan solution and MAP; the effects of chitosan and modified atmosphere, however, seemed independent, as the dipping treatment resulted in a 0.60–2.00 days increase and the MAP caused a further rise of this parameter.

Many studies have shown the high potential of chitosan for preserving fresh fruits and vegetables, but translating this into practice requires optimizing chitosan concentrations for individual crops and improving chitosan coating formulations, for integration with modified atmosphere during transport and storage of perishable commodities.

In order to evaluate the influence of temperature on the mesophilic growth of the uncoated fruits, Q_{10} of stability time was calculated (Fig. 1).

Shelf life of fresh-cut strawberries was primarily affected by temperature; in particular the samples packaged in MA2 were more influenced by temperature than others, showing a Q_{10} of about 3.47, with respect to values of 2.2 in MA1 and 1.39 in CA.

Moreover, the figure shows that the stability time for mesophilic bacteria of samples packaged in MA2 was always

Table 1
Effect of temperature, packaging atmosphere and chitosan coating on the Gompertz parameters of mesophilic bacteria

Packaging	T (°C)	Gompertz parameters[a]				Stability[b] time
		A	μ_{max}	λ	R^2	
CALP[c]	4	3.89±0.10	0.62±0.40	0.46±0.80	0.997	2.77
	8	4.30±0.24	0.71±0.15	0.11±0.30	0.996	2.34
	12	5.50±0.86	**1.24±0.10**	**0.03±0.35**	0.998	1.66
	15	5.64±0.59	1.84±0.72	0.15±0.14	0.997	1.28
CALP+chitosan	4	2.66±0.10	0.37±0.35	0.68±0.76	1.000	3.32
	8	3.48±0.26	0.64±0.16	0.61±0.29	0.999	2.61
	12	4.97±0.84	0.72±0.11	0.00±0.29	0.999	2.54
	15	5.13±0.52	0.84±0.67	0.02±0.10	0.999	2.26
CAHP	4	4.34±0.29	2.28±0.56	0.21±0.08	0.996	0.91
	8	4.77±0.43	2.93±0.93	0.20±0.09	0.997	0.80
	12	5.39±0.23	5.47±0.85	0.15±0.02	0.999	0.51
	15	6.37±0.46	4.65±0.71	0.11±0.52	1.000	0.61
CAHP+chitosan	4	4.50±0.25	1.70±0.47	1.03±0.09	1.000	2.50
	8	3.40±0.58	1.88±1.15	0.27±0.25	0.989	0.93
	12	5.54±0.20	2.05±0.32	0.16±0.05	1.000	1.15
	15	5.90±0.55	5.42±0.58	0.13±0.48	1.000	0.53
MA1	4	2.62±0.20	0.57±0.09	5.16±0.51	1.000	6.85
	8	2.92±0.14	1.05±0.18	4.56±0.23	0.999	5.58
	12	5.09±0.43	1.12±0.52	2.52±1.41	0.996	4.19
	15	6.12±0.28	1.32±0.19	1.30±0.47	0.999	3.00
MA1+chitosan	4	–[d]	–	–	–	–
	8	–	–	–	–	–
	12	3.59±0.14	0.59±0.04	3.34±0.26	0.999	5.58
	15	4.80±0.46	0.64±0.09	1.11±0.97	0.997	3.87
MA2	4	2.72±0.03	0.49±0.02	3.55±0.10	1.000	5.59
	8	3.10±0.10	0.55±0.05	2.13±0.41	1.000	4.20
	12	6.28±0.54	0.90±0.11	0.14±0.14	1.000	2.70
	15	5.96±0.69	2.58±1.30	0.63±0.63	0.993	1.48
MA2+chitosan	4	–[d]	–	–	–	–
	8	–	–	–	–	–
	12	2.83±0.33	0.88±0.30	2.10±1.47	0.999	3.74
	15	6.14±0.45	0.91±0.70	1.17±0.38	1.000	3.62

[a] Gompertz equation parameters: A, maximum bacteria growth attained at the stationary phase log (CFU/g); μ_{max}, maximal growth rate (Δ log CFU/g/days); λ, lag phase (days); R^2, repression coefficient. Data are accompanied by standard errors.
[b] Time corresponding to the maximum of the second time derivative of the Gompertz function.
[c] CALP: control atmosphere with bags at low permeability; CAHP: control atmosphere with bags at high permeability; MA1: modified atmosphere (30% CO_2, 65% N_2, 5% O_2) MA2: modified atmosphere: (80% O_2–20% CO_2).
[d] No growth.

higher than the value of this parameter for CA and MA1 samples. The difference was very important at the lowest temperatures of storage (4 and 8 °C).

The evolution of psychrotrophic bacteria at 12 °C, reported in Fig. 2, exhibited the effectiveness of chitosan treatment in all experimental conditions. In particular, the interaction between chitosan coating and MA1 atmosphere, seemed to be more effective to reduce psychrotrophic cell load: strawberries uncoated in MA1, in fact, showed a lower cell load attained at stationary phase, if compared to the fruits packaged in CA and MA2. Then, chitosan treatment resulted in a further reduction of psychrotrophic cell load. Besides, psychrotrophic bacteria had a very short lag phases under control, independently by the packaging atmosphere; otherwise, this parameter was slightly prolonged under chitosan treatment.

The naturally occurring yeast population was not able to grow at 4 °C in all the packaging atmospheres (Table 2); on the other hand, yeasts had very short lag phases (λ) at 8, 12 and 15 °C. As showed by the parameter A, chitosan displayed a strong inhibition also on this microbial group, above all when combined with the low temperature (Table 2). The presence of chitosan on strawberries did not influence the stability time: no significant difference, in fact, was observed between coated and uncoated strawberries ($p<0.05$).

As regard to lactic acid bacteria, microbial growth was observed only for uncoated strawberries and at the highest temperature. At 4 and 8 °C no growth was observed (data not shown). This result disagreed with the literature evidence; Devlieghere et al. (2004), in fact, reported that lactic acid bacteria were less susceptible to the antimicrobial activity of chitosan than other bacteria.

Finally, moulds were detected only in the samples packed in the high permeability bags and their number seemed to be not affected by chitosan treatment, as bag permeability and O_2 diffusion probably played a predominant role (data not shown).

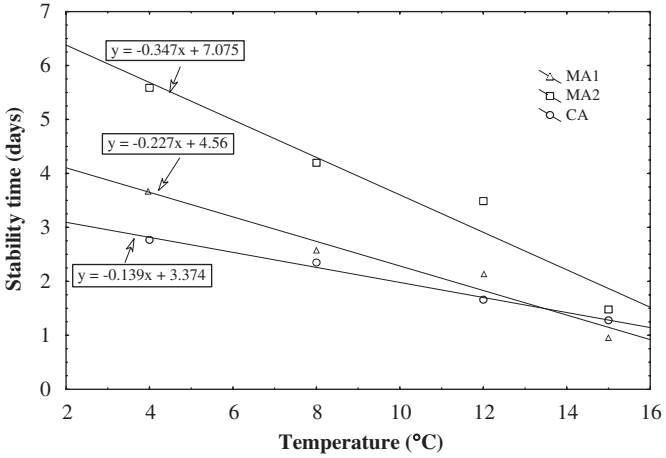

Fig. 1. Influence of the storage temperature on mesophilic bacteria cell load of minimally processed strawberries without chitosan and packed in low permeability bags (CA, air; MA1, modified atmosphere 1—30% CO_2, 5% O_2, 65% N_2; MA2, modified atmosphere 2, 80% O_2, 20% CO_2).

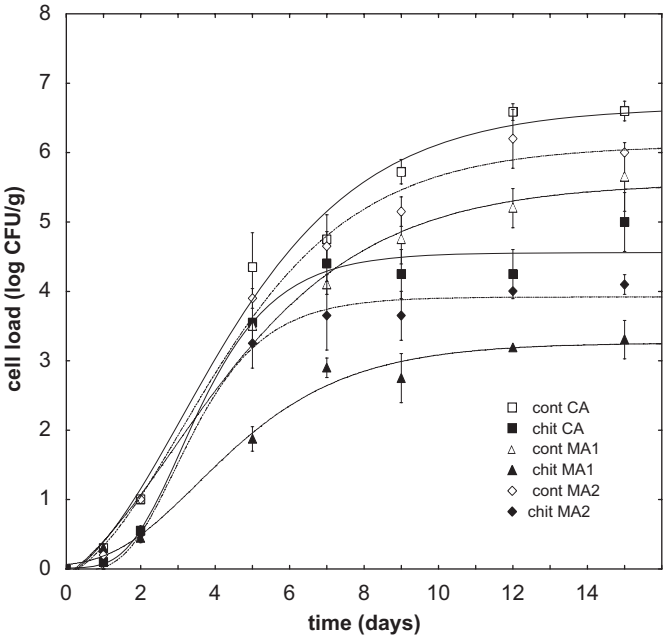

Fig. 2. Evolution of psychrotrophic bacteria cell load within the storage at 12 °C of the samples packed in low permeability bags (CA, air; MA1, modified atmosphere 1—30% CO_2, 5% O_2, 65% N_2; MA2, modified atmosphere 2, 80% O_2, 20% CO_2). Data are the average ($n = 2$) ±standard deviation; the lines are the best fit to the data through the Gompertz equation.

3.2. Effects of atmosphere composition and chitosan coating (at different storage temperature) on the microbial population

The increase in cut damaged surface and availability of cell nutrients, in fresh-cut products, provide conditions that increase the number and types of microbes that can develop.

The identification of naturally occurring bacteria and yeasts, on strawberries packaged in CA and modified atmosphere (MA1 and MA2), was carried out. Under our experimental conditions, the occurring psychrotrophic microflora was mainly represented by *Staphylococcus* spp., independently by chitosan processing. Besides, *Pantoea* spp. (isolated by plants) and *Klebsiella oxytoca* (an opportunist pathogenic microorganism present on fruit and vegetables) were identified, too. The presence of *Staphylococcus* spp. has been extensively reported for minimally processed food and vegetables (Lanciotti et al., 2004); its source is presumably the contamination in the processing line by food handlers, and it does not represent a risk because this microorganism does not compete well with other microorganisms normally present on raw fruit and vegetables. Moreover, none of the strains isolated and identified in this research was positive to the coagulase assay.

Isolation and identification of *Stahpylococcus* spp. from strawberries was reported also by Johannessen et al. (2002), who recovered CNS (coagulase-negative) strains of *Staphylococcus* spp. from the 15% of the analysed samples, belonging to the species *S. epidermidis*, *S. hominis*, *S. xylosus* and *S. capitis*. On the other hand, the ability of *Staphylococcus* spp. to survive and/or grow at low temperature and in acidic foods was reported in the past by Bibac et al. (1996) and Riyaz-Ul-Hassan et al. (2003).

As regard to CA, during the first 5 days of storage, no yeast growth was observed; afterwards, *Kloeckera* spp. was mainly isolated (100%), as reported for cactus pear fruits by Corbo et al. (2004).

Qualitative composition of yeast population underwent to significant changes in the latter days of storage: after 11–15 days, in fact, the predominant microflora was *Cryptococcus laurentii* and *Rhodotorula mucilaginosa*. The prevalence of *Crypto. laurentii* (a casual saprophyte microorganisms of skin) was probably due to an irregular worker manipulation. Moreover, colonization by *Rhodotorula* spp. is usually associated with tropical fruits and ripe apples; the traditional rack and cloth press for juice production is also a major source of contamination and in peer juice *Rhodotorula* was one of the main wild yeasts (Yeeh, 2000).

The isolation and identification of *Rhodotorula* spp. and *Crypto. laurentii* from strawberries could be considered in the light of the biocontrol of spoiling moulds (*Rhizopus* spp. and *Botrytis cinerea*); Zhang et al. (2007a), in fact, reported that a strain of *R. glutinis* could be use as a non-chemical alternative treatment against post-harvest diseases of strawberries. When the yeast was co-inoculated with *B. cinerea* in the wounds of harvested berries, mould growth appeared to be completely inhibited or controlled, for a probable competition for the nutrients.

Similar results were reported for *Cryto. laurentii* against *Rhizopus* spp. by Zhang et al. (2007b).

As observed for CA, yeast population was represented by *Kloeckera* spp. both in MA1 and MA2 controls, stored at 12 and 15 °C; however, some strains of *Candida albicans* were identified. Some differences were recorded in chitosan coated strawberries, as *Candida famata* and *Candida guilliermondii* were the most isolated species.

The growth of lactic acid bacteria was observed only for the samples without chitosan coating and stored at highest temperature (15 °C): *Leuconostoc* spp. was mainly isolated; however, the presence of lactic acid bacteria could be considered positively, since in a complex environment, indigenous or added lactic acid bacteria, which produce inhibitory compounds other than lactic acid, may have competitive advantage that results in suppression of undesirable microflora (Stiles, 1996).

3.3. Effect of chitosan coating on pH, colour, soluble solids and contents of O_2 and CO_2 in the bags

pH of strawberries was very low (about 3.5) and during the storage did not change significantly ($p<0.05$): this parameters seemed to be not influenced by atmosphere packaging, temperature and chitosan treatment (data not shown).

An important parameter to determine the best quality is the soluble solids, which represents an indirect measurement of the hardness of fruits.

Table 2
Effect of temperature, packaging atmosphere and chitosan coating on the Gompertz parameters of yeasts

Packaging	Temperature (°C)	Gompertz parameters[a]				
		A	μ_{max}	λ	R^2	Stability time[b]
CALP	4	–[c]	–	–	–	–
	8	3.81±0.22	0.62±0.16	0.30±0.21	0.997	2.56
	12	6.31±0.41	**1.07±0.14**	**0.19±0.11**	0.999	2.38
	15	6.14±0.40	1.38±0.13	0.14±0.12	0.998	1.78
CALP+chitosan	4	–[d]	–	–	–	–
	8	3.36±0.02	1.95±0.05	2.68±0.12	1.000	3.31
	12	4.62±0.35	0.72±0.13	0.31±0.41	0.997	2.67
	15	5.04±0.35	0.78±0.10	0.18±0.15	0.999	2.56
CAHP	4	5.88±0.14	0.72±0.21	0.21±0.37	0.993	3.21
	8	5.59±0.22	0.88±0.26	0.18±0.26	0.999	2.52
	12	4.77±0.03	4.41±0.09	0.21±0.07	1.000	0.61
	15	6.44±0.13	4.81±0.08	0.14±0.05	1.000	0.63
CAHP+chitosan	4	4.30±0.14	0.59±0.25	0.47±0.34	0.999	3.15
	8	4.04±0.12	0.94±0.08	0.74±0.06	1.000	2.32
	12	4.87±0.14	2.80±0.11	0.22±0.10	1.000	0.86
	15	4.84±0.15	4.41±0.24	0.19±0.04	1.000	0.59
MA1	4	–	–	–	–	–
	8	–	–	–	–	–
	12	5.67±0.24	0.82±0.06	0.20±0.08	0.999	2.74
	15	6.58±0.85	1.12±0.52	0.07±0.02	0.999	2.23
MA1+chitosan	4	–	–	–	–	–
	8	–	–	–	–	–
	12	3.26±0.19	0.73±0.16	0.63±0.23	0.999	2.27
	15	5.06±0.43	1.00±0.14	0.31±0.13	1.000	2.17
MA2	4	–	–	–	–	–
	8	–	–	–	–	–
	12	6.98±0.33	1.05±0.22	0.47±0.15	0.996	2.92
	15	7.32±0.39	1.32±0.17	0.22±0.08	0.999	2.26
MA2+chitosan	4	–	–	–	–	–
	8	–	–	–	–	–
	12	5.52±0.19	0.74±0.17	0.87±0.36	0.999	3.61
	15	6.43±023	0.87±0.06	0.30±0.16	0.998	3.02

[a] Gompertz equation parameters: A, maximum yeasts growth attained at the stationary phase log (CFU/g); μ_{max}, maximal growth rate (Δ log CFU/g/days); λ, lag phase (days); R^2, repression coefficient. Data are accompanied by standard errors.
[b] Time corresponding to the maximum of the second time derivative of the Gompertz function.
[c] CALP: control atmosphere with bags at low permeability; CAHP: control atmosphere with bags at high permeability; MA1: modified atmosphere (30% CO_2, 65% N_2, 5% O_2) MA2: modified atmosphere: (80% O_2–20% CO_2).
[d] No growth.

The hardness of strawberries packaged in CALP (air in low permeability bags) and MA2 atmosphere was not influenced by chitosan treatment; in some conditions, however, chitosan coating seemed to affect it negatively (Table 3).

The interaction chitosan coating-packaging in MA1 improved the tickness of strawberries: in fact, it was observed a reduction of soluble solids and this behaviour was more evident at 4 °C.

At the same temperature, the value of soluble solids for the samples packaged in bags at high permeability did not change significantly ($p<0.05$) (Table 3).

Fig. 3A shows the evolution of parameter a/b within the storage time in CALP packaged samples, both for the inner and the outer sections of the strawberries. For the samples stored at the lowest temperature (4 °C), no differences were observed between strawberries with and without chitosan coating and external and internal of fruit. Otherwise at 12 and 15 °C, a positive influence of chitosan was observed, as one could infer from an higher values of the parameter (data not shown).

The colour of strawberries packaged in MA1 was not influenced by chitosan treatment for all the temperature used (data not shown).

Finally, higher oxygen concentrations (MA2), combined with chitosan coating, seemed to influence positively the colour (Fig. 3B).

The use of high level of O_2 to prolong strawberry shelf life was proposed in the past by several authors; the data of the influence on the colour in the present research, however, are quite different form those reported by the literature. Zheng et al. (2008), in fact, proposed the use of superatmospheric level of O_2 (40–100%) to control the decay of Chinese bayberry, strawberry and blueberry, reporting that high O_2 amounts level did not affect colour changes within the storage, as the fruits underwent to browning (both those packed in air and in modified atmosphere).

In agreement with the paper of Zheng et al. (2008), Allende et al. (2007) reported that the use of MAP with high O_2 content caused a marked decrease of phenols (e.g. of the ellaginin) and a negative influence on the overall quality of the fruits.

Table 3
Evolution of soluble solids (°Brix) during the storage at 4 and 8 °C, and packaging in air (CA), in MA1 (65% N_2–30% CO_2–5% O_2) and MA2 (80% O_2–30% CO_2), in bags at low and high permeability

	CALP (low permeability)				CAHP (high permeability)				MA1				MA2			
	4 °C		8 °C		4 °C		8 °C		4 °C		8 °C		4 °C		8 °C	
	Control	Chitosan	Control	Chitosan	Control	Chitosan	Control	Chitosan	Control	Chitosan	Control	Chitosan	control	chitosan	control	chitosan
t0*	$7.63^{a,b}_{A}$*,***	7.80^{a}_{A}	$7.63^{a,b}_{A}$	$7.80^{a,b}_{A}$	$7.63^{a,b}_{A}$*,***	7.80^{a}_{A}	7.63^{a}_{A}	7.80^{a}_{A}	7.63^{a}_{A}	7.80^{a}_{A}	$7.6_{A}3^{a,b}$	7.80^{a}_{A}	7.63^{a}_{A}	7.80^{a}_{A}	7.63^{a}_{A}	7.80^{a}_{A}
t2	$8.00^{b,c}_{A,B}$	$7.25^{a,b}_{A,B}$	$6.63^{a}_{A,B}$	$8.50^{a,b}_{B}$	8.13^{b}_{A}	6.63^{a}_{A}	8.00^{a}_{A}	6.38^{b}_{A}	$7.63^{a}_{A,B}$	6.25^{d}_{A}	$7.50^{a,b}_{A,B}$	$7.50^{a}_{A,B}$	$7.63^{a}_{A,B}$	$8.25^{a}_{A,B}$	$7.75^{a}_{A,B}$	$8.65^{a}_{A,B}$
t6	$8.88^{c}_{A,B,C}$	$7.63^{a,b}_{C,D}$	$8.50^{a}_{A,B,C}$	$7.13^{a,b}_{A,C}$	$7.38^{a,b}_{A}$	7.75^{a}_{A}	7.75^{a}_{A}	7.63^{a}_{A}	9.75^{b}_{A}	$7.75^{a,b}_{B,C,D}$	$8.75^{a}_{A,B,C}$	9.75^{b}_{A}	6.50^{a}_{D}	$7.50^{a,b}_{C,D}$	$8.25^{a}_{A,B,C}$	$9.25^{b}_{A,B}$
t8	6.50^{a}_{A}	$7.13^{a,b}_{A,B,C,D}$	$6.75^{a,b}_{A,B}$	$8.50^{a,b}_{F}$	$6.75^{a,b}_{A}$	6.63^{a}_{A}	7.38^{a}_{A}	6.75^{a}_{A}	$8.13^{a,b}_{E,F}$	$7.50^{a,d}_{C,D,E}$	$7.38^{a,b}_{B,C,D}$	$7.63^{a}_{D,E}$	$6.88^{a}_{A,B,C}$	$7.25^{a}_{B,C,D}$	$7.75^{a}_{D,E}$	$7.25^{a}_{B,C,D}$
t12	$7.00^{a,d}_{A,B,C}$	6.25^{b}_{A}	$7.13^{a,b}_{A,B,C}$	5.88^{a}	7.00^{a}	6.38^{a}	$8.00^{a}_{C,D}$	$7.13^{b,c}_{A,B,C}$	8.50^{b}_{D}	$8.00^{a}_{C,D}$	$6.88^{a}_{A,B}$	$7.13^{a}_{A,B,C}$	8.50^{a}_{D}	$7.2^{a}_{B,C,D,E}$	$5.50^{a}_{A,E}$	$6.63^{a}_{A,B,C}$
t15	$6.75^{a}_{A,B,C,D}$	$7.38^{a,b}_{B,C,D,E}$	$8.25^{a,b}_{E}$	$6.38^{a}_{A,B}$	$7.63^{a,b}_{A}$	6.50^{a}_{A}	–	–	$8.13^{a}_{A,D,E}$	$6.75^{a,d}_{A,B,C,D}$	$6.25^{a}_{A,B}$	$7.50^{a}_{B,C,D,E}$	$8.00^{a}_{C,D,E}$	$7.2^{a}_{B,C,D,E}$	$5.50^{a}_{A,E}$	$6.63^{a}_{A,B,C}$

* Storage time (days).
** Values in the same column without a common subscript were significantly different (one way ANOVA, Duncan's test: $p<0.05$).
*** Values in the same row without a common superscript were significantly different (one way ANOVA, Duncan's test; $p<0.05$).

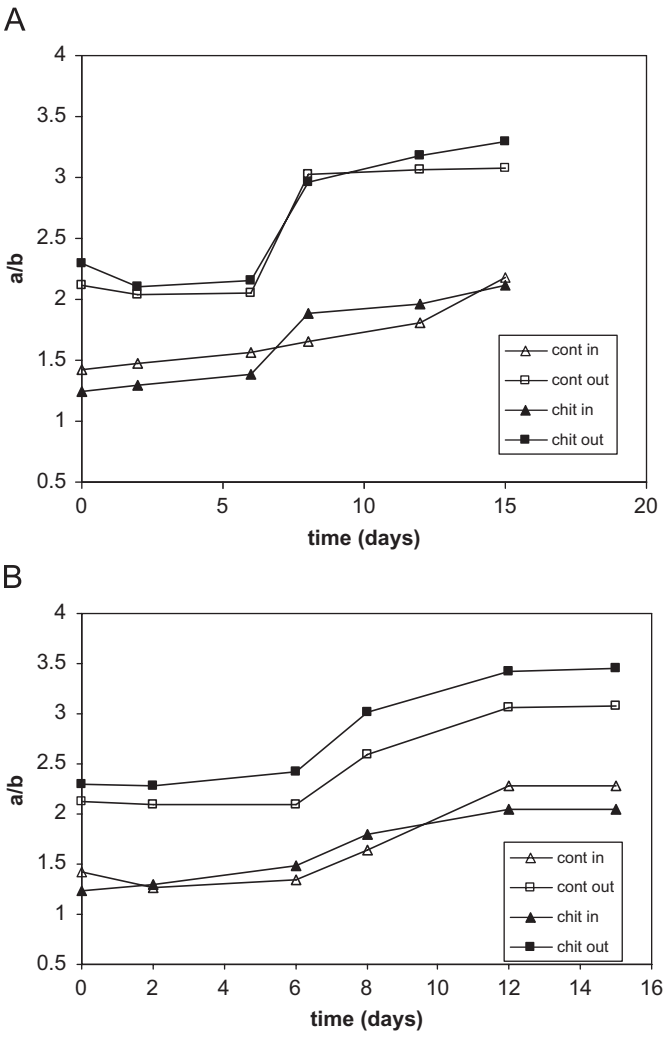

Fig. 3. (A). Evolution of the parameter a/b within the storage time in the samples packed in air in low permeability bags and stored at 4 °C. Data are the average ($n = 5$). Cont, control; chit, chitosan treated strawberries. In, inner section of the strawberries; out, outer section. (B) Evolution of the parameter a/b in the samples packed in MA2 (80% O_2, 20% CO_2) and stored at 8 °C. Data are the average ($n = 5$). Cont, control; chit, chitosan treated strawberries. In, inner section of the strawberries; out, outer section.

This negative effect was not observed in the present research; on the other hand, we recovered a positive influence of O_2 in combination with chitosan, suggesting that the use of a coating could delete the decay of phenolic compounds and the browning the fruits. However, this is only a hypothesis and needs to be confirmed by further investigations.

Fig. 4A reports the evolution of the content of O_2 in the low permeability bags, stored at 4 °C. The treatment with the chitosan did not affect significantly the kinetic of O_2 in the bags and for all the samples a decrease of the gas was observed. In particular, after 2 days O_2 content was $<1\%$ in the samples packed in CA and MA1; on the other hand, in MA2 packaged samples a constant rate of O_2 consumption was recovered and after 8 days its amount in the bags was ca. 50%.

As expected, along with the decrease of the oxygen, an increase of the amount of CO_2 was observed, up to 35–40% and 25% for MA1-MA2 and CA packed samples, respectively (Fig. 4B).

The effect of the storage temperature on the content of CO_2 in CA-packed samples is shown in Fig. 5: An increase of the temperature resulted in drastic rise of respiratory activity of the fruits.

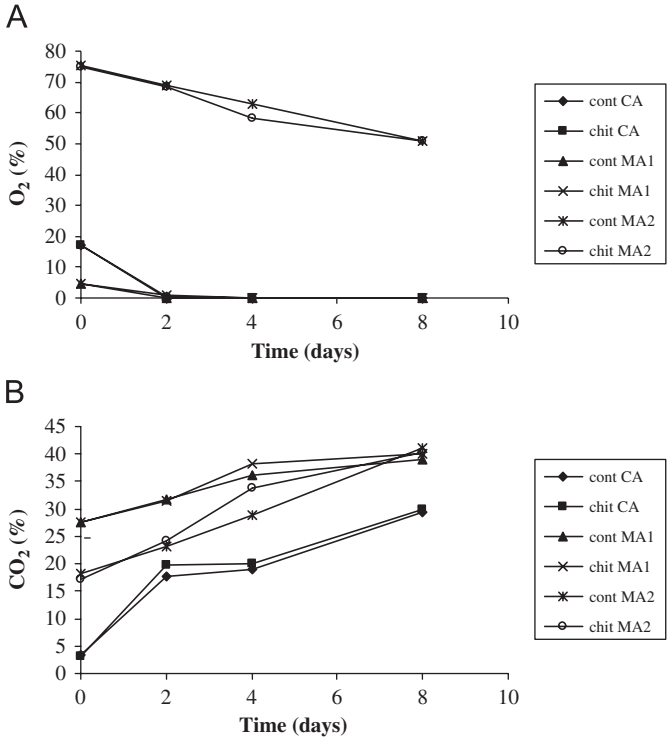

Fig. 4. (A) Content of O_2 (v/v) of the samples packed in low permeability and stored at 4 °C. Data are the average ($n = 2$). (CA, air; MA1, modified atmosphere 1—30% CO_2, 5% O_2, 65% N_2; MA2, modified atmosphere 2, 80% O_2, 20% CO_2). Cont, control; chit, chitosan treated sample. (B) Content of CO_2 (v/v) of the samples packed in low permeability bags and stored at 4 °C. Data are the average ($n = 2$). (CA, air; MA1, modified atmosphere 1—30% CO_2, 5% O_2, 65% N_2; MA2, modified atmosphere 2, 80% O_2, 20% CO_2). Cont, control; chit, chitosan treated sample.

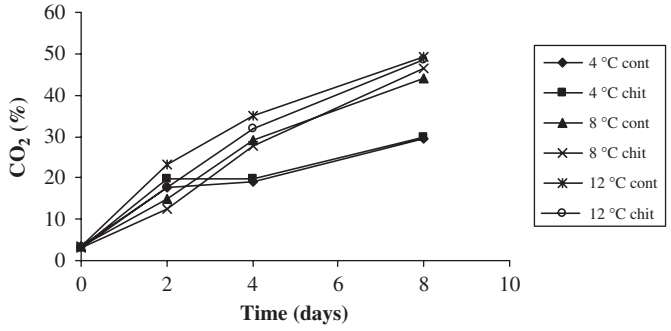

Fig. 5. Effect of the storage temperature on the content of CO_2 (v/v) of the samples packed in air (CA) in low permeability bags. Data are the average ($n = 2$). Cont, control; chit, chitosan treated sample.

4. Conclusions

The results of this paper provided valid information on the growth kinetic of microflora, colonizing minimally processed strawberries.

Chitosan showed a high antimicrobial activity, inhibiting and/or controlling the growth of all considered microbial groups. This inhibition led to an appreciable prolongation of lag phase, a lower cell load and an increase of the stability time.

In the light of the use of edible coatings reported by the literature (1.5% chitosan+calcium gluconate, chitosan+oleic acid, starch and carrageenan, wheat-gluten) (Hernandez-Muñoz et al., 2006; Vargas et al., 2006; Riberiro et al., 2007; Tanada-Palmu and Grosso, 2005), the approach proposed in this research appeared to be more convenient, as the coating with low-molecular-weight chitosan was *invisible* and did not affect the visual appearance and the overall sensorial quality of strawberries.

A second element to keep in mind, is the following: The MAP resulted in a sensible benefit on the microbiological quality, as the use of MA1 and MA2 decreased the population number of all the tested microorganisms. Many authors have demonstrated the positive influence of elevated oxygen concentrations on sensorial characteristics, colour and in inhibiting microbial growth in minimally processed fruit and vegetables (Jacxsens et al., 1999; Amanatidou et al., 2000; Kader and Ben-Yehoshua, 2000; Wszelaki and Mitcham, 2000). Nevertheless, Allende et al. (2002), confirmed that superatmospheric oxygen does not affected all microorganisms in the same way.

The use of bags at high permeability could not be proposed to prolong strawberries shelf-life, because spoilage of strawberries was more precocious than samples packaged in bags at low permeability.

In our experimental conditions, chitosan was not selective against occurring strawberries fresh-cut microflora. pH and thickness values were not changed by chitosan coating, whereas colour was positively influenced by it; this effect was more evident in MA2 packaged samples.

In conclusion, we could recommend the application of chitosan coating to control browning and decay in strawberry fruit in combination with other methods, i.e. low temperature and suitable packaging.

References

Ahvenianen, R., 1996. New approaches in improving the shelf life of minimally processed fruit and vegetables. Trends Food Sci. Technol. 7, 179–187.
Allende, A., Jacxsens, L., Devlieghere, F., Debevere, J., Artes, F., 2002. Effect of superatmospheric oxygen packaging on sensorial quality spoilage, and *Listeria monocytogenes* and *Aeromonas caviae* growth in fresh Processed mixed salads. J. Food Prot. 65, 1565–1573.
Allende, A., Marin, A., Buendia, B., Tomas-Barberan, F., Gil, M.I., 2007. Impact of combined postharvest treatments (UV-C light, gaseous O_3, superatmospheric O_2 and high CO_2) on health promoting compounds and shelf-life of strawberries. Postharvest Biol. Technol. 46, 201–211.
Amanatidou, A., Smid, E.J., Gorris, L.G.M., 1999. Effect of elevated oxygen and carbon dioxide oh the surface growth of vegetables-associated micro-organisms. J. Appl. Microbiol. 86, 429–438.
Amanatidou, A., Slump, R.A., Gorris, L.G.M., Smid, E.J., 2000. High oxygen and high carbon dioxide on the surface growth of vegetable-associated microorganisms. J. Appl. Microbiol. 86, 429–438.
Artés, F., Martinez, J.A., 1996. Influence of packaging treatments on the keeping quality of "Salinas" lettuce. Lebensm.-Wiss. Technol. 29, 664–668.
Baldwin, E.A., Nisperos-Carriedo, M.O., Baker, R.A., 1995. Edible coatings for lightly processed fruits and vegetables. Hort. Sci. 30, 35–38.
Bibac, I., Roy, S., Watada, A.E., Wergin, W.P., 1996. Changes in microbial populations on fresh cut spinach. Int. J. Food Microbiol. 31, 107–119.
Chien, P.J., Sheu, F., Lin, H.R., 2007. Coating citrus (Murcott tangor) fruit with low molecular weight chitosan increases postharvest quality and shelf life. Food Chem. 100, 1160–1164.
Collins, C.H., Lyne, P.M., Grange, J.M., 1989. Collin's and Lyne's Microbiological Methods. Butterworths, London.
Corbo, M.R., Altieri, C., D'Amato, D., Campaniello, D., Del Nobile, M.A., Sinigaglia, M., 2004. Effect of temperature on shelf life and microbial population of lightly processed cactus pear fruit. Postharvest Biol. Tec. 31, 93–104.
Day, B.P.F., 1996. High oxygen modified atmosphere packaging for fresh prepared produce. Postharvest News Inform. Conf. Proc. 7, 31N–34N.
Devlieghere, F., Vermeulen, A., Debevere, J., 2004. Chitosan: antimicrobial activity, interactions with food components and applicability as a coating on fruit and vegetables. Food Microbiol. 21, 703–714.
Devriese, L.A., Pot, B., 1995. The genus *Enterococcus*. In: Wood, B.J.B., Holzapfel, W.H. (Eds.), The Genera of Lactic Acid Bacteria. Blackie Academic and Professional, London, pp. 327–367.
Dong, H., Cheng, L., Tan, J., Zheng, K., Jiang, Y., 2004. Effect of chitosan coating on quality and shelf life of peeled litchi fruit. J. Food Eng. 64, 355–358.
Du, J.M., Gemma, H., Iwahori, S., 1997. Effects of chitosan coating on the storage of peach, Japanese pear, and kiwifruit. J. Jpn. Soc. Hort. Sci. 66, 15–22.
El Ghaouth, A., Ponnampalam, R., Boulet, M., 1991. Chitosan coating effect on storability and quality of fresh strawberries. J. Food. Sci. 56, 1618–1620.
El Ghaouth, A., Ponnampalam, R., Castaigne, F., Arul, J., 1992. Chitosan coating to extend the storage life of tomatoes. Hort. Sci. 27, 1016–1018.

Garcia, M.A., Martino, M.N., Zaritzky, N.E., 1998a. Plasticized starch-based coating to improve strawberry (*Fragaria* × *ananassa*) quality and stability. J. Agric. Food Chem. 46, 3758–3767.

Garcia, B.S.A., Nunes, N.J., Silva, C.S., 1998b. Effect of different pre-freezing treatments on the quality of frozen strawberries variety Chandler Ci. Tecnol. Aliment. 18, 82–86.

Gorris, L.G.M., Peppelenbos, H.W., 1992. Modified atmosphere and vacuum packaging to extend the shelf life of respiring produce. Hort. Technol. 2, 303–315.

Gregory, E., Fridovich, I., 1973. Oxigen toxicity and superoxide dismutase. J. Bacteriol. 114, 1193–1197.

Hammes, W.P., Vogel, R.F., 1995. The genus *Lactobacillus*. In: Wood, B.J.B., Holzapfel, W.H. (Eds.), The Genera of Lactic Acid Bacteria. Blackie Academic and Professional, London, pp. 19–54.

Hernandez-Muñoz, P., Almenar, E., Ocio, M.J., Gavara, R., 2006. Effect of calcium dips and chitosan coatings on postharvest life of strawberries (*Fragaria ananassa*). Postharvest Biol. Technol. 37, 247–253.

Ippolito, A., El Ghaouth, A., Wilson, C.L., Wisniewski, M., 2000. Control of postharvest decay of apple fruit by *Aureobasidium pullulans* and induction of defense responses. Postharv. Biol. Technol. 19, 265–272.

Jacxsens, L., Devlieghere, F., Falcato, P., Debevere, J., 1999. Behaviour of *Listeria monocytogenes* and *Aeromonas* spp., of fresh-cut produce packaged under equilibrium modified atmosphere. J. Food Prot. 62, 1128–1135.

Jiang, Y., Li, Y., 2001. Effects of chitosan coating on postharvest life and quality of longan fruit. Food Chem. 73, 143–159.

Johannessen, G.S., Loncarevic, S., Kruse, H., 2002. Bacteriological analysis of fresh produce in Norway. Int. J. Food Microbiol. 77, 199–204.

Kader, A.A., 1986. Biochemical and physiological bases for effects of controlled and modified atmospheres on fruits and vegetables. Food Technol. 5, 99–105.

Kader, A.A., Ben-Yehoshua, S., 2000. Effects of superatmospheric oxygen levels on postharvest physiology and quality of fresh fruits and vegetables. Postharv. Biol. Technol. 20, 1–13.

Ke, D., Zhou, L., Kader, A.A., 1994. Mode of oxygen and carbon dioxide action of strawberry ester biosynthesis. J. Am. Hortic. Sci. 119, 971–975.

King, A.D., Bolin, H.R., 1989. Physiological and microbiological storage stability of minimally processed fruits and vegetables. Food Technol. 43, 132–135.

Kurtzman, C.P., Fell, J.W., 1998. The Yeasts: A Taxonomic Study. Elsevier, Amsterdam.

Lanciotti, R., Gianotti, A., Patrignani, F., Belletti, N., Guerzoni, M.E., Gardini, F., 2004. Use of natural aroma compounds to improve shelf-life and safety of minimally processed fruits. Trends Food Sci. Technol. 15, 201–208.

Li, P., Barth, M.M., 1998. Impact of edible coating on nutritional and physiological changes in lightly-processed carrots. Postharv Biol. Technol. 14, 51–60.

Ministere de l'Economie des Finances et du Budget. 1998. Marché consommation, Produits vegetaux prets a l'emploi dits de la "IVemme Gamme": Guide de bonnes pratique hygieniques. J. Off. Rép. Française 1621, 1–29.

Moleyar, V., Narasimham, P., 1994. Modified atmosphere packaging of vegetables: an appraisal. J. Food Sci. Technol. 31, 267–278.

Nguyen-the, C., Carlin, F., 1994. The microbiology of minimally processed fresh fruits and vegetables. Crit. Rev. Food Sci. Nutr. 34, 371–401.

Pen, L.T., Jiang, Y.M., 2003. Effects of chitosan on shelf life and quality of fresh-cut Chinese water chestnut. Lebensm.-Wiss. Technol. 36, 359–364.

Porretta, S. 1991. Il controllo della qualità dei derivati del pomodoro. Stazione Sperimentale per l'Industria delle Conserve Alimentari, Parma, pp. 76–83.

Riberiro, C., Vicente, A.A., Teixeira, J.A., Miranda, A., 2007. Optimization of edible coating composition to retard strawberry fruit senescence. Postharvest Biol. Technol. 44, 63–70.

Riva, M., Franzetti, L., Galli, A., 2001. Microbiological quality and shelf-life modeling of ready-to-eat cicorino. J. Food Prot. 64, 228–234.

Riyaz-Ul-Hassan, S., Verma, V., Qazi, G.N., 2003. Microbiological quality of walnut kernels and apple juice concentrate. World J. Microbiol. Biotechnol. 19, 845–850.

Singh, R.P., 1994. Scientific principles of shelf-life evaluation. In: Man, C.M.D., Jones, A.A. (Eds.), Shelf-life Evaluation of Foods. Blackie Academic and Professional, London, pp. 3–26.

Soliva-Fortuny, R., Elez-Martinez, P., Martin-Belloso, O., 2004. Microbiological and biochemical stability of fresh-cut apples preserved by modified atmosphere packaging. Innov. Food Sci. Emerg. Technol. 5, 215–224.

Stiles, M.E., 1996. Biopreservation by lactic acid bacteria. Antonie van Leeuwenoek 70, 331–345.

Tanada-Palmu, P.S., Grosso, C.R.F., 2005. Effect of edible wheat-gluten based films and coatings on refrigerated strawberry (*Fragaria ananassa*) quality. Post-harvavest Biol. Technol. 26, 199–208.

Teuber, M., 1995. The genus *Lactococcus*. In: Wood, B.J.B., Holzapfel, W.H. (Eds.), The Genera of Lactic Acid Bacteria. Blackie Academic and Professional, London, pp. 173–234.

Van der Walt, J.P., Yarrow, D.Y., 1994. Method for isolation, maintenance, classification and identification of yeasts. In: Kreger-van Rij, N.J.W. (Ed.), The Yeasts: A Taxonomic Study. Elsevier, Amsterdam, pp. 45–104.

Vargas, m., Albors, A., Chiralt, A., Gonzalez-Martinez, C., 2006. Quality of cold-stored strawberries as affected by chitosan-oliec acid edible coatings. Postharvest Biol. Technol. 41, 164–171.

Wszelaki, A.L., Mitcham, E.J., 2000. Effects of superatmospheric oxygen on strawberry fruit quality and decay. Postharv. Biol Technol. 20, 125–133.

Yeeh, Y., 2000. *Rhodotorula*. In: Robinson, R.K., Batt, C.A., Patel, P.D. (Eds.), Encyclopedia of Food Microbiology, vol. 3. Academic Press, London, p. 1900.

Zhang, D.L., Quantick, P.C., 1997. Effects of chitosan coating on enzymatic browning and decay during postharvest storage of litchi (*Litchi chinensis* Sonn.) fruit. Postharv. Biol. Technol. 12, 195–202.

Zhang, H., Wang, L., Dong, Y., Jiang, S., Cao, J., Meng, R., 2007a. Postharvest biological control of gray mold decay of strawberry with *Rhodotorula glutinis*. Biol. Control 40, 287–292.

Zhang, H., Zheng, X., Wang, L., Li, S., Liu, R., 2007b. Effect of yeast antagonist in combination with hot water dips on postharvest Rhizopus rot of strawberries. J. Food Eng. 78, 281–287.

Zheng, Y., Zhenfeng, Y., Chen, X., 2008. Effect of high oxygen atmospheres on fruit decay and quality in Chinese bayberries, strawberries and blueberries. Food Control 19, 470–474.

Zwietering, M.H., Jongenberger, I., Rombouts, F.M., van't Riet, K., 1990. Modelling of bacterial growth curve. Appl. Environ. Microbiol. 56, 1875–1881.

〈www.fresh-cut.org〉. [Accessed, October 20th 2007].