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Biopolymer Coatings as Alternative to Modified Atmosphere Packaging for Shelf Life Extension of Minimally Processed Apples

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Abstract: The effect of caseinate/chitosan blend on the shelf life of minimally processed apples was studied. In the first phase of the work, the effect of the biopolymer coating on the respiration rate of the minimally processed apples was studied as function of gas composition (5%, 10%, 21% of O_2 with N_2 as balance at 5 °C) and temperature (5 °C, 10 °C at 5% of O_2 with N_2 as balance). In the second phase, the shelf life of the packed product was studied during storage at 5 °C. The gas composition (O_2%-CO_2%) in the package headspace, relative humidity, pH, hardness, color and antioxidant capacity of the product were monitored after 0, 1, 4, 7, 11, and 14 days. The coating effectively reduced respiration rate of the product when oxygen was over 10%. In the presence of the coating, the reduction of oxygen did not affect the respiration rate. At 5% of O_2, the respiration rate decreased by 50% by changing the temperature from 10 °C to 5 °C. Shelf life study showed that the chitosan—caseinate coating was able to preserve the mechanical properties and the antioxidant capacity of the product during storage by increasing the shelf life by 7 days to 11 days at 5 °C.

Keywords: chitosan; sodium caseinate; respiration rate; mechanical properties; antioxidant capacity; minimally processed apple

1. Introduction

Fruits are key elements of a healthy and balanced diet, providing humans with essential nutrients and bioactive compounds, including vitamins, organic acid, minerals, fiber, and polyphenols. According to the World Health Organization (WHO), to eat plenty of fruit and vegetables (F&V) is one of the 5 keys to a healthy diet. People whose diets are rich in F&V have a significantly lower risk of obesity, heart disease, stroke, diabetes, and certain types of cancer [1]. A wide variety of minimally processed fruits and vegetables (F&V) products has been offered on the market, resulting in an increased consumption and consequently intake of fresh F&V, benefitting the agrifood economy and human well-being. Minimally processed apples are one of the most predominant fruit in the market. Although minimal processing (MP) methods, such as washing, cutting, and modified atmosphere packaging (MAP), have been demonstrated to preserve the freshness of the products, the shelf life of F&V is still limited (5–7 days at 4–5 °C). The quality deterioration of minimally processed apples depends on increased respiration, cut-surface browning, softening, and microbial contamination [2–4]. Enzymatic browning is one of the most important reactions occuring in many fresh-cut fruits and vegetables. It results from oxidation of phenolic compounds catalyzed by polyphenol oxidase (PPO) followed by non-enzymatic spontaneous polymerization of obtained quinones to brown pigments. To control this phenomenon, antioxidant compounds, including ascorbic acid, cysteine and glutathione,

acidulants, and chelating agents have been used in the general fresh-cut food industry and maintaining the commercial value of fresh cut products [5–8]. Moreover, calcium chloride has been used in combination with antioxidant as a firming agent for fruit tissues since it reacts with pectic acid in the cell wall to form calcium pectate, which strengthens molecular bonding between constituents of cell wall [9]. In a previous work, an antioxidant washing solution based on ascorbic acid, citric acid, and calcium chloride has been optimized to preserve the color of minimally processed apples [10]. A way to further prolong product shelf life is to use edible coating. Coatings create a modification of the atmosphere surrounding the fruit, acting as semipermeable barriers that control gas exchange, reduce water loss, and maintain tissue firmness [11]. Developments in packaging technology and edible coatings for foods have shown promising results in extending the shelf-life of fresh-cut fruits and vegetables [12–18]. Coating based on tapioca starch, chitosan, and alginate in combination with antioxidant additive has been used to improve the shelf life of minimally processed apples [19–21]. Until recently, there has been little research that investigated the effect of the combination of biopolymer coating and anti-browning agents for minimally processed products to inhibit enzymatic browning. A combination of carboxymethyl cellulose and ascorbic acid seemed to have a synergistic effect for control of surface browning of fresh-cut apple during storage [22]. In addition, the effect of edible coatings in combination with anti-browning agents on fresh-cut 'Bravo de Esmolfe' apple has been studied [23] and it was reported that based on general and sensory quality characteristics, fresh-cut apples appeared to be better preserved with alginate coating (2%) enriched with eugenol (0.1%) plus dipping in ascorbic acid 0.1%. Chitosan (1%) and ascorbic acid (5%) coating successfully extended the cold storage period for 7 days in fresh-cut apples by maintaining the browning level and preserving microbial and chemical quality [24]. In a previous work, the composition of chitosan/caseinate coating to be applied on minimally processed apples were optimized and its use in combination with an antioxidant treatment based with ascorbic acid, citric acid, and calcium chloride was studied. A blend obtained by mixing 2% of chitosan and 4% of sodium caseinate at ratio 1:1 were able to reduce the respiration rate of the product in air. Moreover, the blend in combination with the antioxidant treatment was the only able to preserve the color of the product up to 7 days at 5 °C [25]. Modified atmosphere packaging is normally used for extending the shelf life of minimally processed product. Until recently, there has been little research that investigated the combined effect of biopolymer coating and gas composition. Thus, the objective of the work was to evaluate the effect of biopolymer coatings and gas composition on product respiration rate. On the base of the results product has been packed by using the best gas composition and the quality of the product was evaluated during storage at 5 °C for 14 days.

2. Materials and Methods

2.1. Preparation of Film Forming Solutions

The chitosan (CH) solution (2%) was prepared by weighing 2 g of chitosan in 100 mL of 1% acetic acid solution (*v/v*) and stirred for 16 h. The sodium caseinate solution (SC) (4%) was prepared by weighing 4 g of SC in 100 mL of PBS buffer and meanwhile agitated with a magnetic stirrer for 4 h. Blend solution (SC/CH = 2), was prepared by adding drop wise the solution of SC to CH with 1:1 ratio. An aliquot of glycerol was added to obtain a glycerol/total solids ratio equal to 0.1.

2.2. Coating Application

Apples were selected for uniform size and appearance, without damages and gently washed with tap water for 3 min. Then, apples were peeled and cut in 16 slices and placed in an anti-browning solution (1% citric acid, 1% ascorbic acid, 1% calcium chloride) for two minutes. Then, samples were immersed in the biopolymer solution for 1 min. Coated and uncoated apple slices were put on a grid and allowed to dry into an air tunnel (Armfield tray drier, Ringwood, Hampshire, UK) at 30 °C for 40 min.

2.3. Respiration Rate Measurement

O_2 consumption (R_{O_2}) and CO_2 production (R_{CO_2}) rates of minimally processed apples were measured using a modified closed system [26]. The product (0.5 kg) were placed in a steel jars (4000 mL) and conditioned at temperature test. The temperature and relative humidity inside the jar were monitored by means of a data logger (Escort Data Login Systems LTD, Modena, Italy).

To measure the respiration rate at equilibrium, samples were conditioned for two hours at desired temperature and then the system was closed to start the gas measurements. Analysis were performed at 5 °C by using gas mixtures containing 5%, 10%, and 21% of oxygen (nitrogen as balance), and at 5% of O_2 (nitrogen as balance) in air at 5 °C and 10 °C.

Gas mixtures were previously produced by a gas distribution firm (SOL S.p.a., Monza, Italy) and packed in certified cylinders. The gas mixture was flushed through a water humidification system at uniform flow rate (1.6 mL·s^{-1}) before being flushed through the jars containing the product. After equilibrium [26], the inlet and outlet valves were closed and the gas composition was monitored over time with an O_2/CO_2 gas analyzer (accuracy of 0.5%), equipped with a needle (Check Mate 9900 O_2/CO_2; Ringsted, Denmark).

The experimental time was 72 h. At constant time intervals (Δt), 3 mL of gas mixture were drawn from the jar head space and analyzed using the gas analyzer.

The free volume (V_f) inside the jar was calculated by using Equation (1):

$$V_f = V - \frac{W}{\rho} \quad (1)$$

where V is the volume of the jar (mL), W is the weight of the apple (kg), and ρ the apparent density of the apple (810 kg·m^{-3}).

R_{O_2} and R_{CO_2}, were determined as follows:

$$R_{O_2} = \left(\frac{dyO_2}{dt}\right) \cdot \left(\frac{V_f}{W}\right) \cdot \frac{1}{100} \quad (2)$$

$$R_{CO_2} = \left(\frac{dyCO_2}{dt}\right) \cdot \left(\frac{V_f}{W}\right) \cdot \frac{1}{100} \quad (3)$$

where yO_2 and yCO_2 are the volumetric concentration (% *v/v*) of O_2 and CO_2, respectively, at time t (h), V_f is the free volume (mL) and W is the weight of the samples (kg). The respiration quotient, RQ, was calculated as follows:

$$RQ = \frac{R_{CO_2}}{R_{O_2}} \quad (4)$$

2.4. Packaging, Storage Condition and Shelf Life Evaluation

Apple samples (88 g) were packed in air by using polypropylene pouch (0.15 m × 0.15 m) and stored at 4 °C for 14 days. After 0, 1, 4, 7, 11, and 14 days, the head space gas composition (O_2%-CO_2%), the moisture content, the color, the pH, the mechanical properties, and the antioxidant capacity were studied as reported in the following sections.

2.4.1. Headspace Gas Analysis

O_2 and CO_2 concentration (% *v/v*) in the package head space were monitored by means of a portable PBI Dansensor A/S (Check Mate 9900 O_2/CO_2; Ringsted, Denmark) analyzer (accuracy ±0.1%), by sampling with a needle 2–3 mL of gas from the package headspace.

2.4.2. Moisture Content, Color and pH

The moisture content was determined by gravimetric method after storing the samples in an oven at 105 °C for a time needed to reach a constant weight (ASTM D2974). The moisture content has been calculated as:

$$UR\ \% = \frac{(P_i - P_f)}{P_i} \cdot 100 \quad (5)$$

where P_i is the weight of sample before and P_f after the oven storage. Three measurements were performed for each sample.

Color was quantified using a colorimeter tristimolous (Minolta Chroma Meter, model CR-300, Osaka, Japan) having a circular measurement area (D = 8 mm). The apple sample were positioned on a blank foil and then measured by placed using the colorimetric sensor. The colorimeter was calibrated using a white standard plate. Total color change (ΔE) was also analyzed (ASTM E1910):

$$\Delta E = \sqrt{\left[(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2\right]} \quad (6)$$

Six measurements were performed on each sample.

To measure the apple pH, ten grams of apple sample were blended for 2 min in 20 mL of deionized water. The pH of the slurry was measured at room temperature using a Cyber Scan pHmeter (Eutech Instuments Pte Ltd., Singapore) (ASTM D2244). Four measurements were carried out on each sample.

2.4.3. Mechanical Properties

Compression test was performed by using a dynamometer Instron (Instron, Model No 4301, Instron Engineering Corp., Canton, MA, USA) has been used equipped with a 1 kN cell. A cylinder (17 cm diameter × 10 cm high) was obtained from apple sample by using a specific tool. The test was performed at 10 mm·min^{-1}. From the curve force-strain, the hardness (N) has been calculated as the maximum force required to compress the sample. Five measurements were performed for each sample.

2.4.4. Antioxidant Capacity

The antioxidant capacity was studied by evaluation of the free radical- scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, according to the method described by Moreira et al. [27] The apple (5 g) was softly crushed with mortar and pestle and 100 µL of the juice was added to 3.9 mL of methanolic DPPH solution (100 µmol). The homogenate was shaken vigorously and kept in darkness for 30 min. Absorption of the samples at 515 nm against a blank of methanol without the DPPH reagent was spectrophotometrically measured (Jasco V-550 UV/VIS Spectrophotometer, Tokyo, Japan). Antioxidant capacity was related to the scavenging activity of the sample extracts towards the DPPH radical, which can be monitored through the decrease in absorbance once the sample extract has been incorporated to the DPPH solution. The antioxidant capacity (TAA%) has been expressed as:

$$TAA\ \% = \left(1 - \frac{A_s}{A_R}\right) \times 100 \quad (7)$$

where A is the absorbance and *s* and R indicated the sample and the reference, respectively. DPPH assays were performed in triplicate.

2.4.5. Experimental Design and Data Analysis

A full factory design was used to study the effect of oxygen concentration and coating on the respiration rate of minimally processed apple. There were three levels of oxygen concentration (5%, 10%, and 21%) and two levels of coating (absence–presence) for a total of 6 samples. The effect of temperature was studied by considering two level of temperature (5 °C, 10 °C) and two level of coating

(absence–presence). A full factorial design was also used to study the effect of time and coating on the quality indices of minimally processed apple. There were six levels of storage time (0, 1, 4, 7, 11, and 14 days) and two levels of coating (absence–presence), thus a total of twelve samples. For all the factors, two-way ANOVA analysis was performed on data to evaluate the effect of independent variables and the interaction effect. Duncan's test and t-test were used to determine significant differences among samples. Significance of differences was defined at $p \leq 0.05$. All statistical analyses were performed using the SPSS software (SPSS Inc. 20.0, Chicago, IL, USA, 2002).

3. Results and Discussions

3.1. Respiration Rate Measurement

Figure 1 shows the respiration rate expressed as the rate of oxygen consumption of the minimally processed apples as function of oxygen concentration (Figure 1A) and temperature (Figure 1B). For control samples, respiration rate changes from 1.79 ± 0.02 to 4.32 ± 0.06 as the oxygen increased from 5% to 21%. The respiratory quotient (RQ) was independent from the oxygen concentration and coating and assumed a constant value of 1.2 ± 0.1 for both control and coated samples. ANOVA analysis highlighted a significant effect of both oxygen and coating on the respiration rate of samples with interaction between the two variables ($p < 0.05$). The biopolymer coating had a significant effect on the respiration rate of the samples at 10% and 21% of oxygen, whereas no significant effect of coating was highlighted at 5% of oxygen. Due to the coating, a reduction of respiration rate of 21% was observed at 10% of oxygen, whereas at 21% of oxygen the reduction was of almost 50%. The reduction of respiration rate due to the reduction of oxygen is significant ($p < 0.05$) for control samples whereas oxygen did not have a significant effect for coated samples. This result can be justified by the barrier propriety of the biopolymer coating that induce a reduction of oxygen available at cellular level. When using edible films and coatings on minimally processed fruit and vegetables, a modified atmosphere can be created around the product reducing the respiration rate and, as a result, the metabolic processes [21]. Thus, coated samples stored in air showed a lower respiration rate of samples without coating due to the modification of oxygen at the cellular level. In fact, biopolymer coating based on chitosan and caseinate due to their hydrophilic nature can act as barrier to nonpolar substances such as oxygen or carbon dioxide. Thus, by using the coating technology in combination with refrigeration temperature the utilization of the modified atmosphere packaging technology can be avoided. Similar results were reported by Qui et al. [20], who showed that chitosan coatings were able to reduce the respiration rate of minimally processed apples stored at 25 °C of almost 50% due to oxygen barrier properties of chitosan coating. A decrease in the respiration rate was also observed for strawberries coated with chitosan—oleic acid coatings, mandarins coated with hydroxypropylmethylcellulose-beeswax-fatty acid film, plums coated with hydroxypropylmethylcellulose—beeswax film and carrots with caseinate—stearic acid coatings [28].

Figure 1B show the combined effect of temperature and coating technology on the respiration rate of samples. As expected, by decreasing the temperature from 10 °C to 5 °C, the respiration rate of samples was decreased of almost 50% for both control and coated samples. The effect of temperature was statistically significant whereas the coating effect was not statistically significant at both temperature when the oxygen is at 5%.

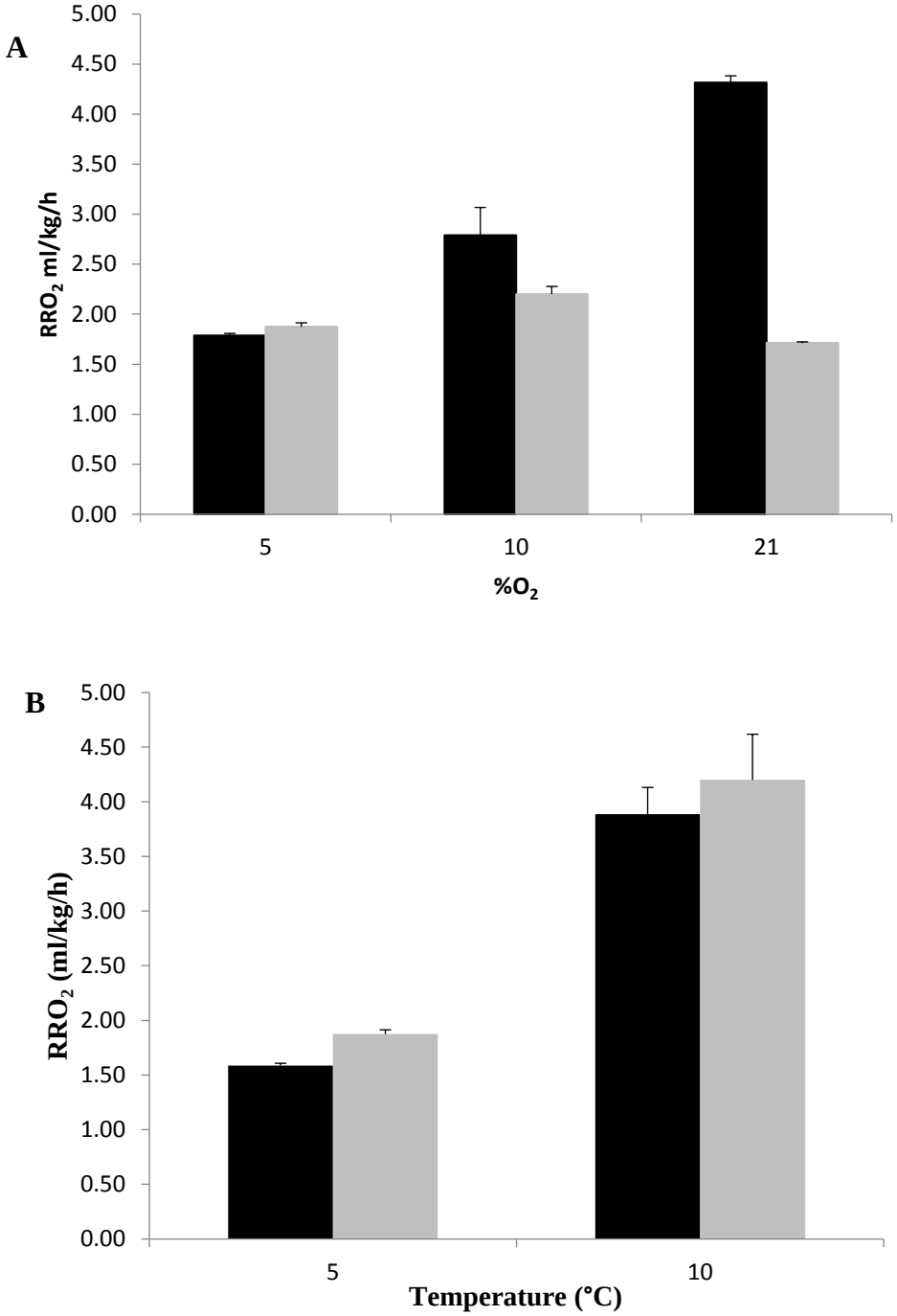

Figure 1. Respiration rate expressed as rate of oxygen consumption (RRO_2) of control sample (■) and coated sample (■) as function of oxygen concentrations at 5 °C (**A**) and as function of temperature at 5% of oxygen (**B**).

3.2. Shelf Life Evaluation

3.2.1. Headspace Gas Analysis

Figure 2 shows the headspace gas composition of minimally processed apples stored at 5 °C for 14 days. As expected, the oxygen decreased during time whereas the carbon dioxide increased due to the respiration rate of the product and the permeability constant of the film. The oxygen changes from 21% to 6.6 ± 0.2% for control samples, whereas for coated samples the equilibrium gas composition was reached after 4 days and the value of oxygen was 11.6 ± 0.1 (Figure 2A). Similar results were obtained

for carbon dioxide (Figure 2B). The equilibrium values were reached after 4 days of storage for coated samples and after 7 days of storage for control samples. For control samples the CO_2 reached a value of 10.4 ± 0.4% whereas for coated samples the value was 7.2 ± 0.5%. These results can be justified by the reduction of respiration rate due to the presence of the coating, in agreement with the results reported in the previous paragraph.

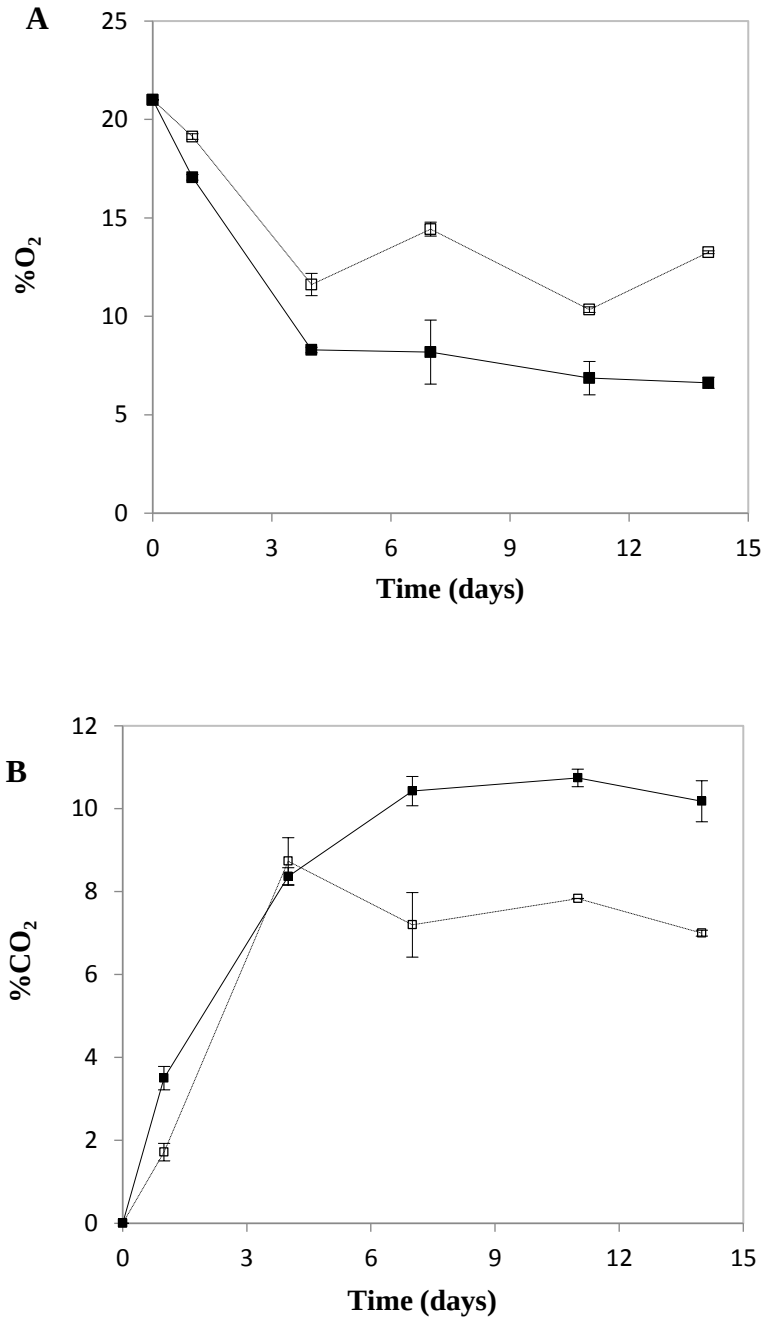

Figure 2. Headspace gas composition (O_2%, (**A**); CO_2%, (**B**)) of minimally processed apples stored at 5 °C for 14 days for control sample (■) and coated sample (□).

3.2.2. Moisture Content, Color and pH

ANOVA analysis showed that the storage time and the coating did not have a significant effect on moisture content of minimally processed apples. The moisture content was constant and equal to 86%. Table 1 reported the colorimetric parameters of the samples at different storage times. For control

samples, L* decreased during storage from 80.6 ± 0.5 to 76.5 ± 0.5 after 14 days of storage with significant effect of time after 1 day of storage. The parameter b* increased from an initial value of 14.8 ± 0.1 to an equilibrium value after 1 day of storage of 17.8 ± 0.3. The parameter a* remained almost constant (−4.4 ± 0.2) with slight variations during storage time, but without a clear trend up to 11 days of storage. After 14 days, the a* value increased up to −3.3 ± 0.1. The parameter ΔE increased during time with significant variation since the first time of storage, reaching after 14 days a value of 7.8 ± 0.1.

Table 1. Color (L*, a*, b*, ΔE) and pH of control and coated samples at different storage time. IQ = quality index.

Sample	IQ	Time (Days)					
		0	1	4	7	11	14
control	L*	80.6 ± 0.5a	75.7 ± 0.5b	75.6 ± 0.4b	75.8 ± 0.6c	75.8 ± 0.2bc	73.5 ± 0.5d
	a*	−4.4 ± 0.2a	−5.0 ± 0.1ab	−5.3 ± 0.5a	−4.53 ± 0.03bc	−4.3 ± 0.5c	−3.3 ± 0.1d
	b*	14.8 ± 0.1a	17.8 ± 0.3b	17.61 ± 0.09b	18.0 ± 1.5b	18.6 ± 0.5b	17.7 ± 0.7b
	ΔE	0 ± 0a	5.6 ± 0.2b	5.5 ± 0.5b	6.8 ± 0.1c	6.3 ± 0.2bc	7.8 ± 0.1d
	pH	3.12 ± 0.01a	3.21 ± 0.01a	4.47 ± 0.05b	4.8 ± 0.1c	5.01 ± 0.02d	5.06 ± 0.07d
coating	L*	80.2 ± 0.1a	78.7 ± 0.3b *	78.5 ± 0.3bc *	78.2 ± 0.8bcd *	77.2 ± 0.3cd *	76.7 ± 1.4d *
	a*	−4.3 ± 0.2a	−4.8 ± 0.2a	−4.3 ± 0.5ab	−3.8 ± 0.5bc	−3.76 ± 0.07bc	−3.6 ± 0.4d
	b*	15.5 ± 0.3a	16.9 ± 0.8b	18 ± 1bc	19 ± 1bcd	20.2 ± 0.5cd *	22.7 ± 0.6d *
	ΔE	0 ± 0a	2.4 ± 0.9b *	3 ± 1b *	4 ± 1c *	5.8 ± 0.4c	8 ± 1d
	pH	3.54 ± 0.02a	3.96 ± 0.06b *	4.08 ± 0.08b *	4.21 ± 0.06c *	4.6 ± 0.2d *	4.94 ± 0.04e

Different letters show a significant effect of time ($p < 0.05$); * significant effect of coating ($p < 0.05$).

The coating showed a significant effect on the color parameters ($p < 0.05$). In particular, in presence of coating the parameter L* decreased slightly reaching a value of 77 ± 1. The coating did not have any effect on the variation of the parameter a*, whereas it had a negative effect on the parameter b* that increased up to a value of 22.7 ± 0.6 after 14 days of storage. However, the effect of the coating was significant on the parameter ΔE, assuring a slowly increasing of this parameter during storage time, with significant effect after 1, 4 and 7 days of storage. Figure 3 shows pictures of the samples after 1, 4, 7 and 11 days of storage. Also, from the image, it is possible to observe the protective effect of the coating on the appearance of minimally processed apples (Figure 3).

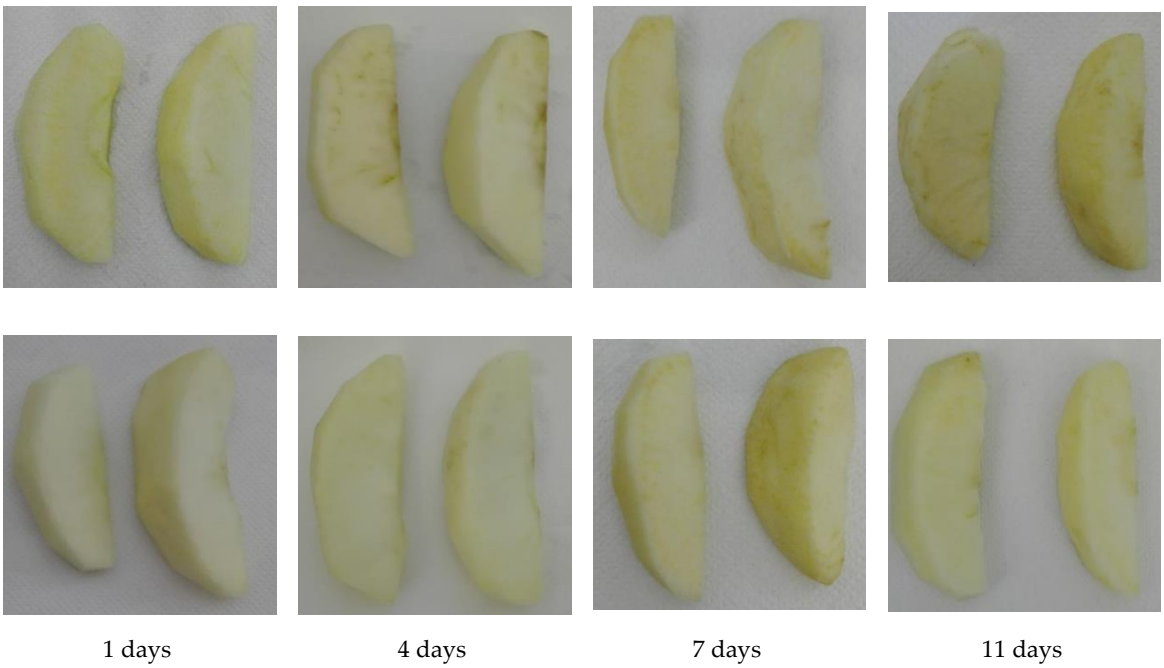

Figure 3. Color change of minimally processed apples during storage for 14 days at 5 °C. Control samples (**high**); coated sample (**bottom**).

The pH of the samples increased during storage time from an initial value of 3.12 ± 0.01 to a value of 5.06 ± 0.07 for control samples (Table 1). The coating has a significant effect on the pH ($p < 0.05$) that increased slightly during storage time, with a significant effect after 1, 4, 7 and 11 days of storage.

3.2.3. Mechanical Properties

During storage time, the hardness of the minimally processed apples decreased from an initial value of 90 ± 5 N to a final value of 76 ± 2 N after 14 days of storage at 5 °C. The coating helped to retard tissue softening during storage. The hardness remained almost constant during storage time (Figure 4). The ANOVA analysis reported a significant effect of the storage time and coating on the hardness parameter. The effect of the coating was significant at all the storage time ($p < 0.05$), except for the time, 11 days ($p > 0.05$). Data agreed with previous results where it was reported a protective effect of the coating on hardness when measured by penetration test [25]. Moreover, similar results were reported by [20] for Fuji apples coated with chitosan.

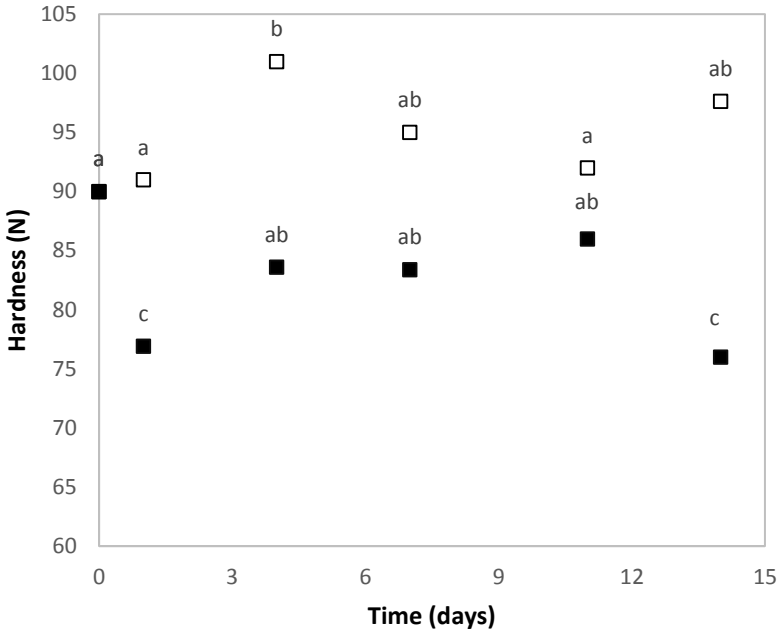

Figure 4. Hardness (N) of minimally processed apples as function of storage time for samples stored 14 days at 4 °C. Control sample (■); coated sample (□). Different letters correspond samples significant effect due to time effect.

3.2.4. Antioxidant Capacity

Figure 5 shows the antioxidant capacity of minimally processed apples during storage at 5 °C. Storage time ($p < 0.05$) and coating ($p < 0.05$) had a significant effect on TAA% with significant interaction between factors ($p < 0.05$). The TAA% decreased from an initial value of 83% to a final value of 53% following a linear decrement. The rate of decrease was slower for coted samples respect to control samples, with significant different between the TAA% values at all storage time except for the samples stored for 14 days of storage. A protective effect of CMC coating enriched with ascorbic acid and calcium chloride has been reported [22]. The protective effect of the coating can be justified by the barrier properties of the coating again oxygen which is an activation factor for oxidation reaction. Moreover, the lower respiration rate in presence of coating inhibits the establishment of an oxidative stress induced by fruit processing and senescence leading to less damage to biological membranes, and lower cell wall hydrolytic enzyme activity [29]. It has also been suggested that ascorbic acid can exert protection by acting as an oxygen scavenger, removing molecular oxygen and avoiding polyphenol oxidase-catalyzed reactions [30]. Thus, it is possible that the protective effect of

the studied coating was due to a combination of contribution of the barrier properties of the coating, and antioxidant activity of the ascorbic acid.

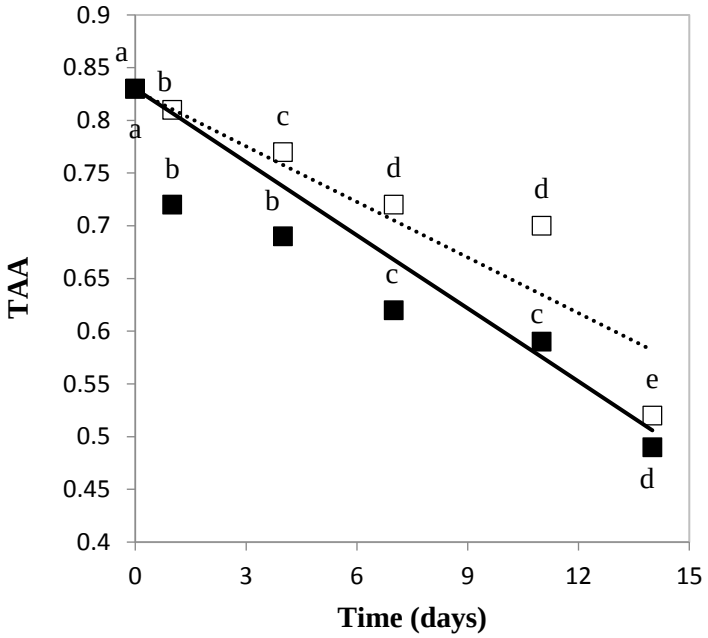

Figure 5. Antioxidant capacity as function of storage time for samples stored 14 days at 4 °C. Control sample (■); coated sample (□). Different letters correspond samples significant effect due to time effect.

4. Conclusions

The results of the present work showed that chitosan–caseinate coating had a protective effect on respiration rate of minimally processed apples with positive impact of physiological parameters. By storage the coated sample in air, the reduction of the respiration rate is equivalent to the reduction obtained with 5% of oxygen. Thus, by using the coating technology the modified atmosphere technology can be avoided. Owing to the barrier properties of the coating to oxygen, a preservation of the antioxidant properties was also highlighted. As general trend by the shelf life study, the coating technologies extend the shelf life of the minimally processed apples from 7 days to 11 days at 5 °C.

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Conflicts of Interest: The authors declare no conflict of interest.

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