

Full Length Research Paper

Combining biocontrol agent and high oxygen atmosphere, to reduce postharvest decay of strawberries

Kraiem Menel¹, Kachouri Faten² and Hamdi Moktar^{1*}

¹Laboratoire Microbial Ecology and Technology, National Institute of Applied Science and Technology (INSAT), B. P. 676, 1080 Tunis, Tunisia.

²Laboratory Microbial Ecology and Technology, School of Food Industries (ESIAT), 53, Alain Savary Street, 1002, Tunisia.

Accepted 27 December, 2011

The use of *Lactobacillus pentosus*, in combination with high oxygen packaging modified atmosphere (O₂MAP) for the preservation of the good quality of strawberries to control fruit decay, was investigated for 4 storage days at 4°C. Fruits without bacterial treatment and high O₂ modified atmosphere packaging (MAP) decreases water loss (82.15%) and fruit decay (66.7%). However, bacterial-treated fruits and high O₂ MAP effects are enhanced giving water loss reduction and a fruit decay preservation at about 33.81 and 14.28%, respectively. The color index, (L x (a/b)), was effectively preserved under the combined bacterial-high MAP with 21.9 for 80% O₂ compared to the control. *L. pentosus* fruit adding was significantly beneficial for quality preservation by enhancing the high O₂ MAP (80% O₂) moulds and yeasts spoilage to 31.47% (p<0.05). The results suggest that bacterial treatments combined with high oxygen packaging atmosphere can be a shelf-life postharvest technique for the strawberry quality preservation. It prolongs the shelf-life of fresh-cut fruit, thanks to the microbial biocontrol agents against fungal pathogen associated to the oxidative stress atmosphere.

Key words: Biological-control, strawberry, high oxygen, lactic acid bacteria, modified atmosphere.

INTRODUCTION

Strawberry (*Fragaria ananassa* Duchesne) is a non-climatic fruit characterized by a short postharvest life estimated to be less than 5 days. It is very prone to rapid dehydration, physiological disorders, bruising, mechanical injuries, and infections caused by several pathogens that can rapidly reduce the quality of the ripe fruit (Sallato et al., 2007).

Microbiological activity on strawberry indicates that yeast produces metabolites, such as, acids, alcohols, and esters which could affect smell and taste depending on their secretion level (Ragaert et al., 2006). Moreover,

microbiologically produced metabolites could be converted into other compounds. Various means of controlling postharvest microbial spoilage of fruit, such as, careful cooling, storage at low temperatures or under controlled atmospheres, and fungicides were used (Eckert and Ogawa, 1988).

The cold storage atmosphere prevents decay development by retarding pathogenic microorganism growth, sporulation and reducing pathogen enzyme activity (Sams and Conway, 1987). But, this temperature storage parameter must be optimized by applying simultaneously other methods to completely prevent decay development and extend the shelf-life of fruit (Spadaro et al., 2002).

Currently, the microbial spoilage fruit infection is treated by synthetic fungicide that favors the proliferation of

*Corresponding author. E-mail: moktar.hamdi@insat.rnu.tn. Tel: +216 98 326 675. Fax: +216 71 704 329.

resistant strain of pathogens (Conway et al., 2004). The resistance increase of fungal pathogens against fungicides had resulted in a significant interest in the development of alternative methods of fruit control (Ragasdale and Sister, 1994).

Biological control was developed as an alternative to synthetic fungicide treatment. A considerable success was achieved after utilizing antagonistic microorganisms to control both preharvest and postharvest diseases (Janisiewicz and Korsten, 2002). Various microbial antagonists have been reported to control several different pathogens on various fruits and vegetables (Fravel, 2005).

A major advantage of using lactic acid bacteria (LAB) as biocontrol agent is that it is generally recognized as safe (GRAS) and usually matches all recommendations for food products (Stiles and Holzapfel, 1997). Moreover, LAB is natural colonizers of fresh fruit and has been previously described as good antagonists of several bacteria and fungi in different food products (Batish et al., 1997; Sathe et al., 2007).

Lactic acid bacteria strains can be used to prevent the development of blue mold. This finding is supported not only by the observed inhibition capacity, but also by other characteristics. In fact, they have a wide growth range, which allows their application under different conditions, including refrigerator temperatures. Moreover, some acid lactic bacteria strains such as *Lactobacillus plantarum* and *Lactococcus lactis* are able to inhibit more than one phytopathogen, which is beneficial for a wide range of plant protection (Trias et al., 2008).

Protection of food from spoilage and pathogenic microorganisms by LAB is done through producing organic acids, hydrogen peroxide, diacetyl (Menssens and De Vugst), antifungal compounds such as fatty acids (Corsetti et al., 1998) or phenyllactic acid (Lavermicocca et al., 2000), and/or bacteriocins (De Vugst and Vandamme, 1994).

The antimicrobial activity was shown in the lactic acid bacteria isolated from a traditional fermented milk "Raib" acting as a barrier to inhibit food spoilage and/or growth of pathogenic microorganisms in foods (Mechai and Kirane, 2008).

It is important to note that there are effective methods which could increase the biological efficacy and inhibit fungal spoilage. Enhancement of biological control could be obtained by combining organic and inorganic additives (Jackson et al., 1991). Droby et al. (2009) affirmed that the biological control is only effective when concentration of the antagonist is reached and the efficacy is not as useful as fungicides. There are many papers about the modest efficiency of the biological biocontrol when applied alone, and researches studies about the efficacy of combining antagonists with other postharvest treatments (Spotts et al., 2002) are appearing in increased number.

The combination of different preservations methods such as modified atmosphere packaging, low temperature

storage or the additional preservation methods may be an excellent way to preserve the original quality attributes of these products (Alzamora, 1998).

Enhancement of the biocontrol activity of antagonist can be obtained when combined with another strawberry treatment, such as, modified atmosphere treatment. Recently, elevated oxygen atmospheres was suggested as an alternative to the traditional low oxygen and high CO₂ modified atmosphere packaging to maintain quality and safety (Day, 1996). Also, high O₂ atmosphere was suggested as an effective method to inhibit the growth of microorganisms and prevent undesirable anoxic fermentation (Kader and Ben Yehoshua, 2000).

The objective of this study is to evaluate the combined effect of the lactic acid bacteria and high oxygen atmosphere treatment on the undesirable microbial strawberries strain and the overall postharvest fruit quality for 4 storage days at 4°C.

MATERIALS AND METHODS

Fruits treatment and storage condition

Strawberries (cv. Camarosa) were harvested at the red ripe stage from a local market and transported to laboratory. After their selection according to size and color uniformity, strawberries were ready to be used for the test.

All samples were stored, after being weighted, under the considered modified atmospheres at 4°C for 4 days. The strawberries were packaged using three packaging models of modified atmosphere: 20, 50 and 80%O₂ balanced with the N₂. All these packaging atmosphere were achieved by using a polyethylene bag, and a gas packaging unit composed of a gas mixer (Witt-Gasetechnik), and a vacuum compensation chamber (mini pack-Torre/Food Division).

Biological material

The bacterial strain used in this study is *L. pentosus* isolated from rabe spontaneous fermented milk grown in Man-Rogosa-Sharpe (MRS) (De Man et al., 1960) at 32°C. *L. pentosus* was prepared in MRS incubated at 32°C for 24 h, and then washed twice by centrifugation (3500 g, 30 min) with 9‰ NaCl solution. The final cell was suspended in 10 ml of 9‰ NaCl solution, resulting in a final bacterial concentration ranging from 1.3 to 3.1 × 10⁶ CFU/ml. The bacterial cocktail was then immediately sprayed on the strawberries under a slight agitation to maximize the bacteria adherence to the fruit surface. After that, the fruit were placed on a sterile sealed polyethylene bag (100 g) and kept at 4°C under different modified atmospheres.

Visual decay assessment, weight loss and water content

The visual decay of the strawberries was measured by weighing fruit with visible mycelium growth and with damaged surface due to softening and bruising. Fruit decay was expressed as a percentage of fruit showing decay symptoms (Zheng et al., 2007).

The weight loss due to transpiration and respiration of the fruit was measured by weighing the fruit each day of the experiment, and expressed as a percentage of the original weight of the packaged fruit (Zhang et al., 2008).

To estimate the water content, strawberry were heated at 100°C for 2 h in a Universal Oven (Memmert UNB500). Water content was expressed using the weight after heating as a percentage of the initial weight.

Color surface measurement, total titrable acidity (TAA) and pH

The fruit surface color intensity was measured with a hand-held Tristimulus reflectance colorimeter (Spectrocolorimetre mobile color-test/ Erichsen SARL). Color was recorded using the CIE - L* a* b* uniform color space (CIE-Lab), where L* indicates lightness, a* indicates chromaticity on a green to red axis, and b* chromaticity on a blue to yellow axis (Francis, 1980). These recorded color values (a* and b*) or some of their combinations should be considered as the physical parameters to describe the visual color degradation. Ahmed et al. (2002) founded out that a representation of visual quality in terms of total color may be more relevant. This is why they founded that L* (a*/b*) or (L* a* b*) are the best combination (Ahmed et al., 2004). The intensity or the saturation color was expressed by Chroma (C*) ($C^* = [a^{*2} + b^{*2}]^{1/2}$).

The titrable acidity was determined by titration with NaOH (0.1 N) to an end point of pH = 8.1. The results were calculated as percent of citric acid (Nunes et al., 1995).

The pH of the puree was determined using a pH-meter (WTW, pH/oxi 340i) standardized to pH 4 and 7.

Microbiological analysis

After opening the packages, 30 g of each package was aseptically added into a stomacher bag and diluted with a peptone saline solution (8.5 g NaCl/L + 1 g peptone/L). A dilution series was made (1:10) and the microbial levels of the packaged vegetables were determined after plating each one onto the appropriate media. Lactic acid bacteria were counted on MRS agar plate (MRS-agar, Oxoid) after 3 days of incubation at 30°C. Sorbic acid (Sigma) was added to MRS-agar at 1.4 g/L to prevent growth of yeast and moulds. The aerobic psychrotrophic count was determined on plate count agar (PCA, oxoid), then incubated at 22°C for 5 days. The yeasts and mould count was enumerated on yeast glucose chloramphenicol agar (YGC-agar) after 3 days of incubation at 30°C (Van der Steen et al., 2002).

Statistical analysis

The storage experiment was conducted in triplicate. The statistical analyses were performed by ANOVA and the Student's t-test. The results were expressed as means ± SE to show variations in the different experiments. Difference was considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Overall quality

At 20% O₂ storage condition, we observed a low water content which can be reported to be due to fruits packaging (Table 1). In fact, packaging enhanced loss of firmness during cold storage by affecting loss of cell wall integrity (Amarante et al., 2002). At 20% O₂ there was no interesting effect observed for the refrigeration. Cordenunsi et al. (2005) demonstrated that reducing the

storage temperature is an effective way to extend the strawberry shelf-life maintaining the fruits edible for additional days. However, temperature can also affect some ripening-related processes, which in turn can improve both sensorial and nutritional value.

The 20% O₂ strawberries atmosphere showed the greatest loss of weight at about 91.17 and 32.35%, respectively for the control and the bacterial treated samples, at the end of the storage period (Figure 2). Van der Steen et al. (2002) reported in similar works that the resulting loss after 3 days of storage was due to fungal respiration translated into H₂O production. Wszelaki and Mitcham (2000) reported in comparable published works that low O₂ modified atmosphere prevents the strawberries *Botrytis* growth. These are major factors which affect the fruit quality causing up to 50% of loss.

The bacterial fruit spray reduced the weight loss through coating the fruit surface and avoiding the higher water loss. This fact is explained by the construction of a hydrophobic film forming a continuous matrix around the product reported to be bacterial exopolysaccharides (Sánchez-González et al., 2010).

The LAB adding prevented water loss for high oxygen storage atmosphere condition especially for the 80% O₂ which improves the water preservation at about 43.33% compared to 20% O₂ (Figure 2). The high oxygen atmosphere enhanced the water fruit preservation compared to the control about 59.67 and 33.81%, respectively for 50 and 80% O₂. The LAB fruit adding provided the preservation of the fruit quality due to the bacterial inhibitory metabolites that avoid growth which cause damage in the fruit surface.

The non bacterial treated fruit decay was also affected by the applied O₂ treatments and reached 14.28% for 80% O₂ compared to 20% O₂ (Figure 1). A similar example of decay suppression under high O₂ level during cold storage at 5°C was also observed in blueberries by Zheng et al. (2003). The enriched atmospheres with O₂ ≥ 60 kPa were effective in inhibiting strawberry fruit decay during storage tanks to the oxygen atmosphere concentration toxicity (Zheng et al., 2007).

Fruit quality was preserved after the bacteria adding for the entire storage oxygen atmosphere 33.3, 57.14 and 66.7% for 20, 50 and 80% O₂, respectively (Figure 1).

The fruit quality preservation is a result of the water loss reduction. Also, the oxidative stress may affect synthesis and accumulation of some volatile compounds associated with respiratory metabolism, including fermentative metabolites such as acetaldehyde, ethanol and ethyl acetate (Wszelaki and Mitcham, 2003).

The adding of bacterial to the fruit prevented decay for all oxygen atmospheres especially for the 80% O₂ which experienced a decrease of about 58.3% compared to the control (Figure 1). Bacterial coatings associated to the modified packaging atmosphere could delay fruit senescence, by decreasing both respiration rate and water losses, which could be explained by the changes in the mechanical response and color development during

Table 1. Effect of the oxygen atmosphere concentration on strawberries color parameters (a and b) and indice ((L*(a/b) and Chroma value), during 3 days of storage at 4°C.

Day	% O ₂	a		b		L*(a/b)		Chroma value	
		Control	Inoculated	Control	Inoculated	Control	Inoculated	Control	Inoculated
Day 0		39.37±0.23	42.54±0.16	20.25±0.16	22.30±0.83	72.64±1.38	76.27±2.54	44.27±0.77	48.03±1.65
Day 1	20	35.15±0.36	38.72±0.23	21.51±0.17	22.33±0.16	60.15±1.63	66.12±1.94	41.21±0.75	44.70±0.67
	50	39.43±0.11	43.33±0.09	21.95±0.38	25.98±0.39	72.11±1.86	73.10±1.39	45.13±0.91	50.52±0.80
	80	34.42±0.09	37.99±0.27	20.75±0.22	22.66±0.83	53.28±1.04	61.88±0.93	40.19±0.06	44.23±0.53
Day 2	20	37.64±0.34	31.10±0.11	23.87±0.11	25.08±0.27	52.19±0.89	43.79±2.54	44.57±1.46	39.95±0.46
	50	39.84±0.08	31.07±0.32	25.02±0.27	25.21±0.75	65.22±0.64	44.49±2.65	47.04±1.84	40.01±1.99
	80	35.63±0.13	34.73±0.15	21.58±0.16	22.34±0.82	56.19±0.82	56.37±3.85	41.66±1.36	41.29±1.28
Day 3	20	35.02±0.24	21.24±0.15	21.05±0.18	13.91±0.55	53.79±0.91	33.82±2.48	40.86±1.25	25.39±0.66
	50	32.28±0.43	17.45±0.03	22.85±0.08	12.25±0.36	53.70±0.75	35.88±1.84	39.55±0.98	21.32±1.75
	80	36.14±0.61	24.41±0.05	22.55±0.12	22.55±0.26	19.50±0.15	23.13±2.89	42.60±1.32	33.23±1.35

* Data expressed as means ±SE of triplicate essays.

storage (Garcia et al., 1998).

pH and acidity

The lactic bacteria added to the fruit promoted the pH decrease compared to the control at the end of the test for all the studied storage atmospheres. The main observed values for the 80% O₂ atmosphere are in accordance with an increase at about 10.9% for the bacterial adding while they reached only 9.2% for the control (Table 1). According to El-Ziney (1998), media acidification was explained by the bacterial metabolites. Lactic and acetic acids are the main products of the fermentation of carbohydrates by lactic acid bacteria, generally recognized as safe agents for the preservation of foods. In their steps, Kalt et al. (1999) attribute this media acidity to the higher

strawberry acid ascorbic preserved tanks to the intracellular compartmentalization of ascorbic acid and phenols. At the end of the test, after the lactic acid bacteria adding, we observed a fruit acidity increase of about 18.2% 80% O₂ (Table 1). Acidity increase and pH decrease were reported for lactic acid bacteria oxygen toleration under a high oxygen concentration atmosphere. In fact, Gram negative bacteria are better protected from the toxic effect of oxygen metabolites because of the synthesis of an outer lipo-polysaccharide layer that traps active molecular oxygen (Dahl et al., 1989).

We also observed a noticeable rise of acidity of 56% for the 80% O₂ atmosphere compared to the control 20% O₂. Pérez and Sanz (2001) founded similar results in strawberry exposed to a high oxygen atmosphere (90 kPa O₂) before day 4 compared to fruit held in air.

Color surface measurement

During the storage time, the chroma decreased slightly for the bacterial treated strawberries compared with the control (Table 2). It was reduced slightly ($P < 0.05$) for the bacterial high oxygen treated sample compared to the control. At the end of storage, the chroma value decreased significantly for the bacterial treated samples and was about 31% while it was only 4% for the control at 80% O₂ atmosphere. The color fruit pre-preservation was explained by the LAB pH decrease. In fact, according to Brouillard (1988), at the pH of 5 to 7, the anthocyanins are unstable and are quickly decolorized due to hydration at the 2-position of the anthocyanidin skeleton. Result confirmed by Keller (1984) who associated the fruit color to the media pH reporting that the acid induced gelatin of pectin at pH levels is lower than 3.5.

Table 2. Effect of the oxygen atmosphere concentration on the strawberries physiochemical parameters: pH, acidity and dry matter, during 3 days of storage at 4°C*.

Day	Treatment	pH		Acidity (% citric acid)		Dry matter (%)	
		Control	Inoculated	Control	Inoculated	Control	Inoculated
Day 0		3.58±0.33	3.58±0.33	0.82±0.08	0.82±0.08	0.34±0.85	0.34±0.85
Day 1	20%O ₂	3.48±0.46	3.65±0.01	0.80±0.05	0.84±0.08	0.42±1.45	0.35±0.79
	50%O ₂	3.57±0.91	3.42±0.06	0.77±0.02	0.79±0.09	0.45±1.20	0.40±0.07
	80%O ₂	3.54±0.94	3.39±0.05	0.81±0.88	0.78±0.02	0.54±0.18	0.3±0.02
Day 2	20%O ₂	3.36±1.34	3.43±0.05	0.77±0.54	0.79±0.25	0.47±0.74	0.45±0.16
	50%O ₂	3.34±1.39	3.38±0.02	0.72±0.67	0.78±0.16	0.61±0.38	0.55±0.24
	80%O ₂	3.33±1.34 ^a	3.35±0.03	0.77±0.30	0.77±0.33	0.87±0.03	0.59±0.91
Day 3	20%O ₂	3.33±1.88	3.34±0.01	0.77±0.44	0.77±0.04	0.65±0.36	0.45±0.08
	50%O ₂	3.29±1.32	3.23±0.02	0.71±0.58	0.74±0.26	0.84±0.75	0.62±0.74
	80%O ₂	3.25±1.44	3.19±0.15	0.75±0.19	0.73±0.12	1.23±0.88	0.68±0.09

* Data expressed as means ±SE of triplicate essays.

We observed an interesting effect of the bacterial fruit adding on the color preservation (color index), especially at the last storage day with an improvement of 37, 33 and 20 and 50% O₂, respectively (Table 2). The fruit browning surface protection was reported for the ascorbic acid. This matches the finding of Dias and Weimer (1998) who explained that lactobacilli produce free thiols which reduce glutathione as an alternative to ascorbic acid.

In this study, the controls' samples showed noticeable fruit browning surface during 4 storage days. The mechanism involved in browning inhibition by antioxidant is explained by LAB treatments. Similar results was reported by Lin and Yen (1999) who demonstrated the antioxidative activity of the intracellular lactic acid bacteria-free extract with an inhibition rate of ascorbate oxidation in the range of 7 to 12%.

Oxidative browning is usually caused by the enzyme polyphenol oxidase (PPO) which in the presence of O₂ converts fruits and vegetables phenol compound into dark colored pigments. Ascorbic acid can prevent PPO-mediated cut surface discoloration by reducing pH surface, and further slowing browning reaction (Beaulieu and Gorny, 2004). This finding reinforced the hypothesis of the protective effect of the LAB on the natural ascorbic acid fruit content, and on the syntheses of new antioxidant compounds.

Microbiological counting

The *L. pentosus* spray on fruit reduced significantly the natural spoilage of strawberries at the end of test (Figure 3) for all the atmospheric studied conditions ($p < 0.05$).

Under the 20% O₂ atmosphere, we observed a contamination decrease at about 19.23%. El-Tarabily and Sivasithamparam (2006) suggested several modes of action listed as follows: The biocontrol of antagonistic yeast, such as, competition for nutrients and space, production of cell wall-degrading enzymes, production of antifungal diffusible and volatile metabolites, and induction of host resistance and mycoparasitism. The observed antimicrobial effect was reported for hydrogen peroxide accumulation. Comparable results was reported by Gonzalez-Flecha and Demple (1995) concerning the LAB hydrogen peroxide production. They explain that under the aerobic condition, the LAB endogenously synthesized H₂O₂ may permeate the cell membrane and be released easily out of the cells until its accumulation.

The non bacterial treated sample experience an interesting spoilage reduction by rising the oxygen storage atmosphere (Figure 3) compared to the control (20% O₂). It was about 5.12 and 19.79% for the following atmosphere, 50 and 80% O₂, respectively. These results indicate the important oxidative role on the fruit spoilage safety. Day (1998) suggested in a similar study that high O₂ modified atmosphere was very effective at inhibiting microbial growth and reducing decay of the fruit. The high oxygen atmosphere storage condition combined with the bacterial treatment decreased the moulds and yeast contamination rate at about 19.71 and 38.98% for the following atmosphere, 50 and 80% O₂, respectively (Figure 3). These results suggest that high oxygen concentration improve the bacterial activity of *L. pentosus* against the intact strawberries postharvest spoilage.

Numerous articles have reported the positive effect of high oxygen atmosphere on the postharvest fruit spoilage

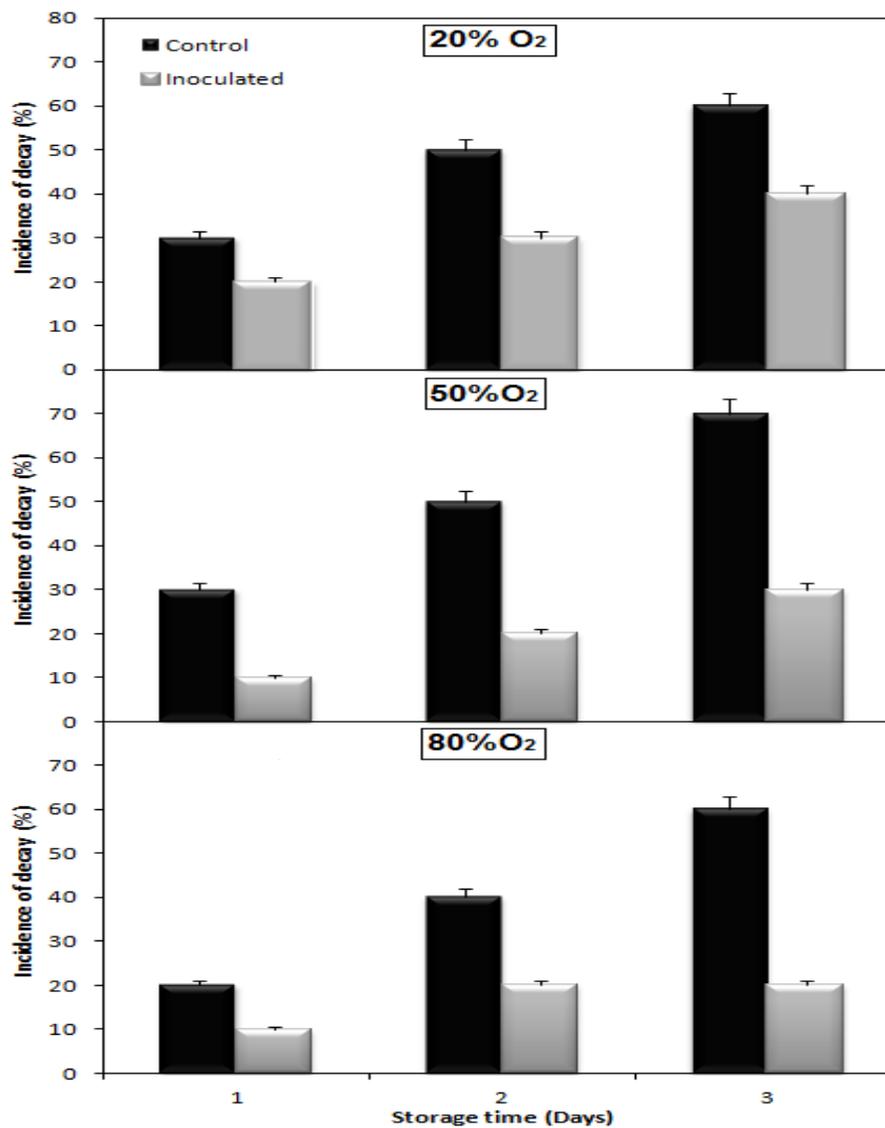


Figure 1. Effect of the oxygen atmosphere concentration on the strawberries decay indices, during 3 days of storage at 4°C. Without lactic acid bacteria (■) and with lactic acid bacteria adding (■). Bars represent standard deviations of the means.

reduction. The results suggested the use of 80 kPa O₂ to reduce significantly mycelium growth and the amount of moulds spores (Van der Steen et al., 2002) and the use of 100 kPa to reduce effectively mycelium growth than 15 kPa CO₂ or air after 14 days at 5°C (Wszelaki and Mitcham, 2000).

The number of aerobic psychrotrophic bacteria increased after the bacterial fruit treatment, under the different studied MAP reaching 11.35% at the end of the test for the 20% O₂ atmosphere. However, it attained 16.89 and 18.49% for the 50 and the 80% O₂ atmosphere, respectively compared to the control (Figure 3). This increase was a logic result to the lactic acid bacteria proliferation in this adequate environment under high

oxygen atmosphere. The aerobic psychrotroph also observed an increase after the rise of oxygen atmosphere. It was about 49 and 63% for the 50 and the 80% O₂, respectively compared to the 20% O₂. The number of psychrotrophic bacteria increase was reported to the lactic acid bacteria oxygen tolerance. Supporting this idea, Condon (1987) demonstrated that the hydrogen peroxide sensitive lactic bacteria exposed to a sub-lethal of oxygen concentration became capable of growth in the presence of a lethal concentration of hydrogen peroxide. The bacterial fruits adding enhanced the natural lactic microbiota of the strawberries under the 20% O₂ atmosphere reaching a rate of 16.22% (Figure 3). Without the bacterial treatment oxygen atmosphere

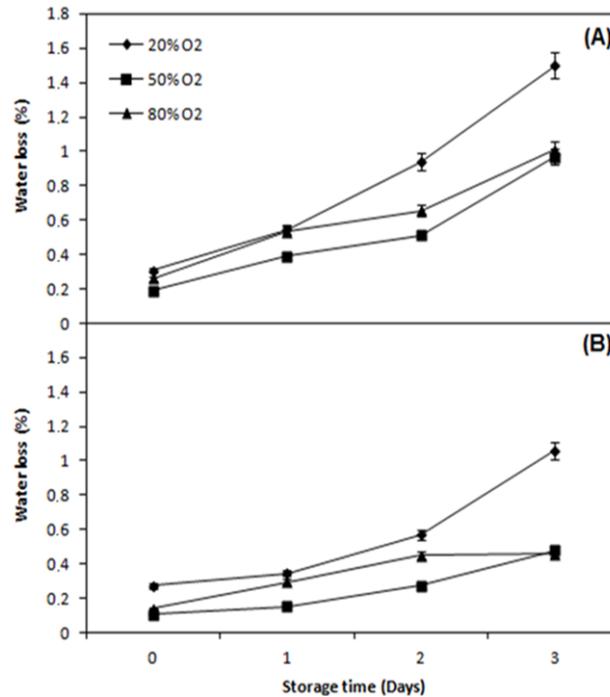


Figure 2. Effect of the oxygen atmosphere concentration on the strawberries water loss (%), during 3 days of storage at 4°C. Without lactic acid bacteria (A) and with lactic acid bacteria adding (B). Error bars indicate one standard error.

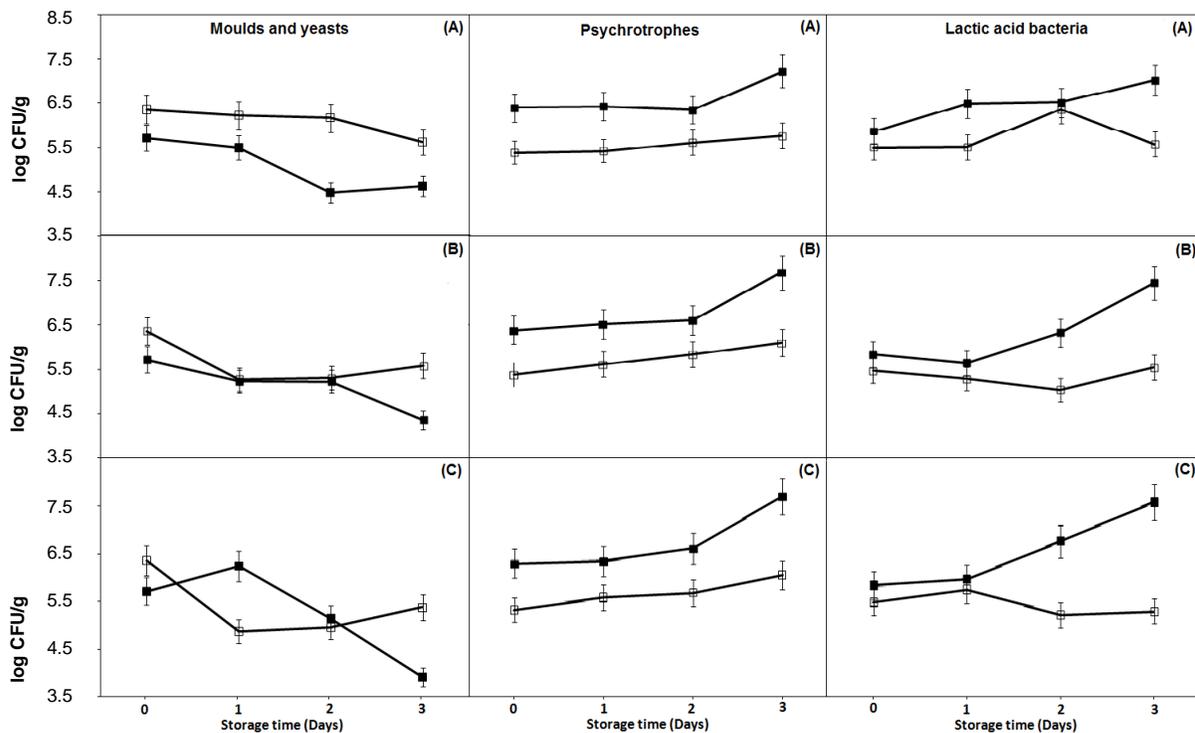


Figure 3. Effect of the oxygen atmosphere concentration on the microbial strawberries fruits evolution, during 3 days of storage at 4°C. Without lactic acid bacteria (□) and with lactic acid bacteria adding (■). The modified atmosphere condition: 20% oxygen atmosphere (A), 50% oxygen atmosphere (B) and 80% oxygen atmosphere (C). Error bars indicate one standard error.

induced a slight increase in the lactic bacterial fruit content, especially for the 80% O₂ atmosphere with a rate of 16.49% compared to 20% O₂ (control). Beside, the LAB count increased under high MAP at about 21.21 and 23.17% for the following atmosphere, 50 and 80% O₂, respectively (Figure 3). *L. pentosus* tolerates the oxidant stress and enhances the effect of high oxygen storage atmosphere. Similar results have been reported by Higuchi et al., (2000) showing that NADH oxidase LAB induced by O₂ in an oxygen-tolerant strain contain two types of enzyme activity, one forming H₂O₂ and the other H₂O.

Conclusion

The strawberry high oxygen modified atmosphere treatment has affected the water loss, the fruit decay and the microbial profile. But, it did not affect color and pH after four storage days at 4°C. The *L. pentosus* bacterial combination with the oxygen MAP treatment preserved the fruit quality and microbiology especially for 80% O₂ atmosphere, where the decrease of the moulds and yeast and the increase of the lactic acid bacteria enhance the color and the water fruit preservation, resulting in fruit quality preservation at the end of the test. This bacterial-high MAP postharvest storage technique must be thoroughly studied in order to replace the un-safety chemical techniques widely applied.

REFERENCES

- Ahmed J, Shivhare US, Kaur M (2002). Thermal colour degradation kinetics of mango puree. *Int. J. Food Prop.*, 5: 359–366.
- Ahmed J, Shivhare, US, Raghavan, GSV (2004). Thermal degradation kinetics of anthocyanin and visual colour of plum puree. *Eur. Food Res. Technol.*, 218: 525–528.
- Higuchi SM, Tapia MS, Welti J (2000). New strategies for minimally processed foods. The role of multi-target preservation. *Food Sci. Technol. Int.*, 4:353–361.
- Amarante C, Banks NH, Max S (2002). Effect of preharvest bagging on fruit quality and postharvest physiology of pears (*Pyrus communis*). *New Zeal. J. Crop Hort.*, 30: 99-107.
- Batish VK, Roy U, Lal R, Grover S (1997). Antifungal attributes of lactic acid bacteria. *Crit. Rev. Biotechnol.*, 17: 209-225.
- Beaulieu JC, Gorny JR (2004). Fresh-cut fruits. In: Gross KC, Wang CY, Saltveit ME (3rd eds) *USAD Handbook N.66 The commercial of fruits, vegetables and florist and nursery stocks*, Washington DC Agriculture Research Service, USA.
- Brouillard R (1988). Flavonoids and flower colour. In: Harborne JB (eds) *The flavonoids. Advances in research since 1980*. Chapman and Hall, London, pp. 525-538.
- Condon S (1987). Responses of lactic acid bacteria to oxygen. *FEMS. Microbiol. Rev.*, 46: 269-280
- Conway WS, Levrentez B, Janisiewicz WJ, Blodgett AB, Saftner RA, Camp MJ (2004). Integrating heat treatment, biocontrol and sodium bicarbonate to reduce potharvest decay of appel caused by *Colletotrichum acutatum* and *Penicillium expansum*. *Postharvest Biol. Technol.*, 34: 11-20.
- Cordenunsi BR, Genovese MI, do Nascimento JRO, Hassimotto NMA, dos Santos RJ, Lajolo FM (2005). Effects of temperature on the chemical composition and antioxidant activity of three strawberry cultivars. *Food Chem.*, 91: 113-121.
- Corsetti A, Gobetti M, Rossi J, Damiani P (1998). Antimould activity of sourdough lactic acid bacteria: identification of a mixture of organic acids produced by *Lactobacillus sanfrancisco* CB1. *Appl. Microbiol. Biotechnol.*, 50: 253–256.
- Dahl TA, Midden WR, Hartman PE (1989). Comparison of killing of Gram-negative and Gram-positive bacteria by pure singlet oxygen. *J. Bacteriol.*, 171: 2188-2194
- Day BPF (1996). High oxygen modified atmosphere packaging for fresh prepared produce. *Postharvest News and Information*, 7: 1N–34N.
- Day BPF (1998). Novel MAP a brand new approach. *Food Manuf.*, 73: 22–24.
- De Man JC, Rogosa M, Shape ME (1960). A medium of cultivation for *Lactobacillus*. *J. Appl. Bacteriol.*, 23, 130-135.
- De Vugst L, Vandamme E J (1994). *Bacteriocins of lactic acid bacteria, microbiology genetics and applications*, Blackie Academic and professional, London.
- Dias B, Weimer B (1998). Conversion of Methionine to Thiols by Lactococci, Lactobacilli, and Brevibacteria. *Appl. Env. Microbiol.*, 64: 3320-3326.
- Droby S, Wisniewski M, Wilson C (2009). Twenty years of postharvest biocontrol research: Is it time for a new paradigm. *Postharvest Biol. Technol.*, 52: 137-145.
- Eckert JW, Ogawa JM (1988). The chemical control of postharvest diseases: deciduous fruits, berries, vegetables and root/ tuber crops. *Ann. Rev. Phytopathol.*, 26: 433– 469.
- El-Tarabily KA, Sivasithamparam K (2006). Potential of yeasts as biocontrol agents of soil-borne fungal plant pathogens and as plant growth promoters. *Mycoscience*, 47: 25–35.
- El-Ziney M (1998). Antimicrobial activity of lactic acid bacteria metabolites: The role of lactic acid enterocin 5701 and reuterin. PhD dissertation, University of Gent, Belgium.
- Francis FJ. (1980). Colour quality evaluation of horticultural crops. *HortScience*, 15: 58–59.
- Fravel DR (2005). Commercialization an implementation of biocontrol. *Ann. Rev. Phytopathol.*, 43: 337–359.
- Garcia JM, Medina RJ, Olias JM (1998). Quality of strawberries automatically packaged in different plastic films. *J. Food Sci.*, 6: 1037–1041.
- Gonzalez-Flecha B, Demple B (1995). Metabolic sources of Hydrogen Peroxide in Aerobically Growing *Escherichia coli*. *J. Biol. Chem.*, 270: 13681-13687.
- Jackson AM, Whipps JM, Lynch JM, Bazin MJ (1991). Effects of some carbon and nitrogen sources on spore germination, production of biomass and antifungal metabolites by species of *Trichoderma* and *Gliocladium virens* antagonistic to *Sclerotium cepivorum*. *Biocontrol Sci. Technol.*, 1: 43-51.
- Janisiewicz WJ, Korsten L (2002). Biological control of postharvest diseases of fruits. *Ann. Rev. Phytopathol.*, 40: 411–441.
- Higuchi M, Yamamoto Y, Kamio Y (2000). Molecular biology of oxygen tolerance in lactic acid bacteria: Functions of NADH oxidases and Dpr in oxidative stress. *J. Biosci. Bioeng.*, 90: 484-493.
- Kader AA, Ben-Yehoshua S (2000). Effects of superatmospheric oxygen levels on postharvest physiology and quality of fresh fruits and vegetables. *Postharvest Biol. Technol.*, 20: 1–13.
- Kalt W, Forney CF, Martin A, Prior RL (1999). Antioxidant capacity, vitamin C, phenolics, and anthocyanins after fresh storage of small fruits. *J. Agric. Food Chem.*, 47: 4638–4644.
- Keller J (1984). Pectin. In: D.L. Downing (eds) *Gum and Starch Technology 18th Annual Symposium*. New York State Agri. Exp. Sta. Special Report 53, USA.
- Lavermicocca P, Valeria F, Evidente A, Iazzaroni S, Corsetti A, Gobetti M (2000). Purification and characterization of novel antifungal compounds by sourdough *Lactobacillus plantarum* 21 B. *App. Environ. Microb.*, 66: 4084-4090.
- Lin MY, Yen CL (1999). Antioxidative Ability of Lactic Acid Bacteria. *J. Agric. Food Chem.*, 47: 1460–1466.
- Mechai A, Kirane D (2008). Antimicrobial activity of autochthonous lactic acid bacteria isolated from Algerian traditional fermented milk "Raïb". *Afr. J. Biotechnol.*, 7: 2908-2914.
- Menssens W, De Vugst L (2002). Inhibitory substances produced by Lactococci isolated from sourdoughs. *In. J. Food Microbiol.*, 72: 31-43.
- Nunes MCN, Brecht JK, Sargent SA, Morais AM (1995). Effects of

- delays to cooling and wrapping on strawberry quality (cv. Sweet Charlie). *Food Control*, 6: 323-328.
- Pérez AG, Sanz C (2001). Effect of high oxygen and high carbon dioxide atmospheres on strawberry flavor and other quality traits. *J. Agric. Food Chem.*, 49: 2370–2375.
- Ragaert P, Devlieghere F, Loos S, Dewulf J, Van Langenhove H, Debevere J (2006). Metabolite production of yeasts on a strawberry-agar during storage at 7°C in air and low oxygen atmosphere. *Food Microbiol.*, 23: 154–161.
- Ragasdale NN, Sister HD (1994). Social and political implications of managing plant disease with decreased availability of fungicides in the United States. *Ann. Rev. Phytopathol.*, 32: 51-58.
- Sallato BV, Torres R, Zoffoli JP, Latorre BA (2007). Effect of boscalid on postharvest decay of strawberry caused by *Botrytis cinerea* and *Rhizopus stolonifer*. *Span. J. Agric. Res.*, 5: 67-68.
- Sams CE, Conway WS (1987). Additive effects of controlled atmospheres storage and calcium chloride on decay, firmness retention, and ethylene production in apples. *Plant Dis.*, 71: 1003–1005.
- Sánchez-González L, Gonzalez-Martinez C, Chiralt A, Chafer M (2010). Physical and antimicrobial properties of chitosan–tea tree essential oil composite films. *J. Food Eng.*, 98: 443-452.
- Sathe SJ, Nawani NN, Dhakephalkar PK, Kapadnis BP (2007). Antifungal lactic acid bacteria with potential to prolong shelf-life of fresh vegetables. *J. Appl. Microbiol.*, 103: 2622-2628.
- Spadaro D, Vola R, Piano S, Gullino ML (2002). Mechanisms of action and efficacy of four isolates of the yeast *Metschnikowia pulcherrima* active against postharvest pathogens on apples. *Postharvest Biol. Technol.*, 24: 123–134.
- Spotts RA, Cervantes LA, Facteau TJ (2002). Integrated control of brown rot of sweet cherry fruit with a preharvest fungicide, a postharvest yeast, modified atmosphere packing, and cold storage temperature. *Postharvest Biol. Technol.*, 24: 251–257.
- Stiles M, Holzapfel W (1997). Lactic acid bacteria of foods and their current taxonomy. *Int. J. Food Microbiol.*, 36: 1-29.
- Trias R, Baneras L, Montesinos E, Bados E (2008). Lactic acid bacteria from fresh fruit and vegetables as biocontrol agents of phytopathogenic bacteria and fungi. *Int. Microbiol.*, 11: 231-236.
- Van der Steen C, Jacxsens L, Devlieghere F, Debevere J (2002). Combining high oxygen atmospheres with low oxygen modified atmosphere packaging to improve the keeping quality of strawberries and raspberries. *Postharvest Biol. Technol.*, 26: 49–58.
- Wszelaki AL, Mitcham EJ (2000). Effects of superatmospheric oxygen on strawberry fruit quality and decay. *Postharvest Biol. Technol.*, 20: 125–133.
- Wszelaki AL, Mitcham EJ (2003). Effect of combinations of hot water dips, biological control and controlled atmospheres for control of gray mold on harvested strawberries. *Postharvest Biol. Technol.*, 27: 255–264.
- Zhang H, Ma L, Wang L, Jiang S, Dong Y, Zheng X (2008). Biocontrol of gray mold decay in peach fruit by integration of antagonistic yeast with salicylic acid and their effects on postharvest quality parameters. *Biol. Control.*, 47: 60–65.
- Zheng Y, Wang SY, Wang SY, Zheng W (2007). Changes in strawberry phenolics, anthocyanins, an antioxidant capacity in response to high oxygen treatments. *Food Sci. Technol. Leb. Wiss. Technol.*, 40: 49–57.
- Zheng Y, Wang CY, Wang SY, Zheng W (2003). Effect of high-oxygen atmospheres on blueberry phenolics, anthocyanins, and antioxidant capacity. *J. Agric. Food Chem.*, 51: 7162–7169