Trends in Food Science & Technology 46 (2015) 320–330

Contents lists available at ScienceDirect

Trends in Food Science & Technology

journal homepage: http://www.journals.elsevier.com/trends-in-food-science-and-technology

Modified atmosphere packaging for shelf life extension of fresh-cut apples

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ARTICLE INFO

Article history:
Available online 16 June 2015

Processing steps, such as cutting and peeling, increase the respiration rate and ethylene production of apples, quickening senescence phenomena with effects on texture, color and flavor. Modified atmosphere packaging (MAP) and antioxidant pre-treatments are used to control the decay of fresh-cut apples during shelf life. MAP has become a widely used food preservation technique as it minimally affects fresh product characteristics. The purpose of this paper was to discuss the influence of conventional (O_2, N_2 and CO_2) and alternative (Ar and N_2O) MAPs as well as the interaction between anti-browning treatment and MAPs on ethylene production, firmness, browning, off-flavor and sensory characteristics, contextualizing the results obtained in a case study on 'Golden Delicious' apple slices developed within the Stayfresh project. The packaging under conventional modified atmospheres, characterized by low O_2 level (1 and 5%), and the alternative mix Ar + CO_2 successfully preserved the firmness of apple slices during all refrigerated storage limiting the ethylene production, even if the preserving efficacy of MAP resulted almost completely nullified by the dipping treatment, which caused a structural breakdown. MAPs were not able to control the enzymatic browning if not combined with an anti-browning dipping treatment. It was highlighted the key role of sensory analysis in finding the best combination between MAP, anti-browning treatment and shelf life time. The contrasting results among the various research groups could be reasonably also due to the different periods and temperatures of shelf life.

Introduction

Modified atmosphere packaging (MAP) is a technique used for prolonging the shelf life of fresh or minimally processed food (Sandhya, 2010). MAP has become a widely used food preservation technique as it minimally affects fresh product characteristics and it is perceived as a natural and additive-free technique by consumers (Day, 1996). This preservation technique consists of substituting the air surrounding the food in the package with an atmosphere with a different composition. So the shelf life of perishable products like meat, fish, fruit and vegetables could be prolonged with MAP delaying the physic-chemical changes related to quality loss of the product. The atmosphere composition in the package depends mainly on the type of product, but also on packaging materials and storage temperature. As fruit and vegetables are respiring products, the interaction between the product and the packaging material is particularly important. The permeability of the packaging film for O_2 and CO_2 has to be suitable for the specific product respiration rate in order to establish a balanced modified atmosphere in the package. This packaging technology is the most commonly used for fresh-cut products. For packaging vegetables and fruit, the modified atmosphere usually consists of a lower O_2 level and a higher CO_2 level than those of air, which slow down the normal respiration rate, prolonging the shelf life of the product. Current low oxygen MAP techniques may suffer from some inherent disadvantages. Novel high O_2 MAP is an innovative development that has been shown to be particularly effective in inhibiting enzymic discoloration, preventing anaerobic fermentation reactions and inhibiting both aerobic and anaerobic microbial growth (Day, 2001). It is hypothesized that active oxygen radical species damage vital cellular macromolecules and thereby inhibit microbial growth when oxidative stresses overwhelm cellular protection systems (Day, 2001). The microbiological aspect will not be in-depth analyzed in this review.

The three main conventional gases used in modified atmosphere packaging are CO_2, O_2 and N_2. They could be used singularly or in combination with the aim of safely extending product shelf life as well as preserving optimal sensory properties of the food.

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http://dx.doi.org/10.1016/j.tifs.2015.06.002
0924-2244/

Recently, there has been a great interest in the potential benefits of using argon and other noble gases for MAP applications (Mostardini & Piergiovanni, 2002; Spencer, 1995). Argon is a tasteless and odorless gas, denser than nitrogen, which is included in the positive list of food additives (E-398) and can be used as balance gas in MAP (Spencer, 2005). Another "new" packaging gas, nitrous oxide (N_2O), has been allowed for food use in the EU (Day, 1996). Noble gases such as argon are in commercial use for products such as coffee and potato-based snack products. Day (2001) reported that a broad range of patents claim that argon, compared to N_2, can more effectively inhibit enzymic activities, microbial growth and degradative chemical reactions in selected perishable foods. More specifically, an Air Liquide patent for fresh produce applications claims that Ar and N_2O are capable of extending shelf life by inhibiting fungal growth, reducing ethylene emission and slowing down sensory quality deterioration (Fath & Soudain, 1992). However, literature data on their application and benefits for the shelf life of fresh-cut fruit is limited (Char *et al.*, 2012; Cocci, Rocculi, Romani, & Dalla Rosa, 2006; Rocculi, Romani, & Dalla Rosa, 2004; Rocculi, Romani, & Dalla Rosa, 2005). Recently, Wu, Zhang, and Wang (2012) have been coupling the use of argon with high pressure, finding that the inhibiting effect of argon was enhanced in such conditions because of the formation of a glass hydrate of the inert gas which, inhibiting the enzymatic reactions, reduced the metabolism of the product. This combination could be an effective method for improving quality of fresh-cut apples at cold storage conditions.

This review is dedicated to MAP technology, applied to extend the shelf life of fresh cut apples with special attention to fruit physiology, quality characteristics such as texture (firmness), color (browning) and aroma (off-flavor formation) and their relationship with sensory characteristics. The interaction between MAP and dipping treatment, applied to preserve color and texture, was also debated in depth.

Ethylene

Ethylene is a natural plant hormone and plays a central role in the initiation of ripening and it is physiologically active in trace amounts (0.1 $\mu L\ L^{-1}$). On the strength of the role of ethylene in the ripening process, fruit can be divided into two groups (Lelièvre, Latché, Jones, Bouzayen, & Pech, 1997):

1 fruit that could produce large amount of ethylene, which promotes their ripening, defined "climacteric", such as tomato, peach, apple, banana and kiwifruit;
2 fruit that produce only low basal amount of ethylene during ripening and are insensitive to exogenous ethylene, defined "non-climacteric", such as grape, strawberry, watermelon, pineapple and citrus.

For both fruit and vegetables the stress caused by technological steps, such as peeling and cutting, produces a physiological response with increased ethylene production and respiratory activity, with effects being observed very rapidly, often within minutes to a few hours (Toivonen & Brummel, 2008). The effect of wounding, caused by the several stages of producing fresh-cut products, can be more evident in climacteric fruit, for which wound-induced ethylene promotes ripening and softening. In climacteric fruit, wound-induced as well as exogenous ethylene may cause the same effect on tissue, causing a hastening of ripening and softening (Toivonen & Brummel, 2008). The general effects of ethylene are usually detrimental to fruit quality (Saltveit, 1999); therefore, its concentration or activity should be minimized to lengthen product shelf life. Being a climacteric-type fruit, apple results very sensitive to ethylene. It has been reported that low O_2 atmospheres and elevated CO_2 levels synergically act to reduce ethylene production and respiration rates, but could not completely stop senescence and tissue breakdown (Soliva-Fortuny & Martín-Belloso, 2003a). On the other hand, super-atmospheric levels of O_2 fail in extending the storability of fresh fruit and vegetables by enhancing the ethylene production (Kader & Ben Yehoshua, 2000).

The inhibition of ethylene production under anaerobic or low O_2 conditions has been observed by many authors, suggesting that oxygen participates in the conversion of 1-amino-cyclopropane-1-carboxylic acid (ACC) to ethylene (Yang, 1981). The action of CO_2 is complex. At low concentrations, carbon dioxide activates the biosynthesis of ethylene via the latter's role as co-factor for 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase (Smith & John, 1993). At high concentrations, carbon dioxide may stimulate the activity of ACC oxidase while inhibiting its synthesis (Cheverry, Sy, Pouliquen, & Marcellin, 1988). A complete inhibition of ethylene in fresh-cut 'Fuji' apples stored under oxygen-free conditions was found by Gil, Gorny, and Kader (1998), Anese, Manzano, and Nicoli (1997) reported that fresh-cut 'Golden Delicious' apples packed in air showed a maximum headspace ethylene concentration between 8 and 16 days of storage, while those packed in presence of N_2 as well as of CO_2/N_2 (80% CO_2 + 20% N_2) did not produced ethylene in detectable amounts. Soliva-Fortuny, Oms-Oliu, and Martín-Belloso (2002) confirmed that ethylene production of fresh-cut 'Golden Delicious' apple slices is almost completely inhibited in N_2-packaged samples. In contrast, if air was used as initial atmosphere, ethylene dramatically increased just after processing and packaging, reaching a maximum concentration, 100-fold higher than in N_2-packaged slices, at 10 days of storage. Successively ethylene concentration decreased in all bags, showing that its synthesis slowed down or even ceased after the first 10 days of storage. Furthermore, Soliva-Fortuny, Ricart-Coll, and Martín-Belloso (2005), evaluating the internal atmosphere of 'Golden Delicious' apple cubes packaged under 0 kPa O_2 and 2.5 kPa O_2/7 kPa CO_2, found that ethylene concentration increased dramatically from the first hours after processing in both cases. Infact the ethylene developed similarly in the apple tissue for all treatments as long as the O_2 was available for its biosynthesis. The maximum concentrations of ethylene, induced by wounding response, were reached between the first and the second week of storage. However, after the first week, internal ethylene concentrations resulted substantially lower in fresh-cut apples packaged under 0 kPa O_2 initial atmospheres, whereas the different oxygen permeability of the plastic material (15 and 30 $cm^3\ O_2\ m^{-2}\ bar^{-1}\ day^{-1}$) did not have a significant effect on ethylene evolution. The same authors confirmed that packaging under restrictive O_2 conditions limited ethylene response. Moreover they observed that ethylene response of the apple tissue, also after re-exposure to atmospheric conditions, was slightly lower for samples stored under initial conditions of anoxia. Rojas-Graü, Grasa-Guillem, and Martín-Belloso (2007) also confirmed that ethylene production was noticeably higher in 'Fuji' apple slices packaged under initial air atmosphere, achieving a maximum concentration of 60 $\mu L\ L^{-1}$ after one week of storage. In contrast, apple slices packaged under 2.5 kPa O_2 + 7 kPa CO_2 produced less ethylene and reached a maximum peak of 35 $\mu L\ L^{-1}$. Contrasting results have been obtained when the influence of the dipping step in antibrowning agents, applied to preserve color, on ethylene production was investigated. Soliva-Fortuny *et al.* (2002) demonstrated that dipping in ascorbic acid and calcium chloride did not entail noticeable changes in ethylene concentrations compared with samples where a dip was not carried out. Rojas-Graü *et al.* (2007) confirmed that ethylene production of fresh-cut apples was not affected by ascorbic acid but the ethylene evolution was significantly reduced by N-acetylcysteine. Previously, Gil

et al. (1998), analyzing the interaction between ascorbic acid dips with headspace atmospheres with or without oxygen on 'Fuji' apple slices, found that this type of dipping reduced ethylene production only when air atmosphere packaging was used.

The results obtained by Cortellino, Rizzolo, and Gobbi (2015) for 'Golden Delicious' apple slices were in agreement with literature data as it is shown in Fig. 1. Ethylene headspace concentration (Fig. 1A) was noticeably higher in apples packaged under air atmosphere, achieving a maximum concentration after 11 days of cold storage at 4 °C with slightly different quantity in the two different experiments (51 and 58 μmol/kg). In contrast, apple slices packaged under either nitrogen (99% N_2 + 1% O_2) or in a mixture of conventional gases (90% N_2 + 5% O_2 + 5% CO_2) produced a very small quantity of ethylene (max 15 μmol/kg) with a similar trend during cold storage. To our knowledge there are no studies on the effect of alternative modified atmosphere on the evolution of ethylene during cold storage; so, the Cortellino *et al.* (2015) trial is the first research which tried to fill this gap. 'Golden Delicious' apple slices packaged in an argon and nitrous oxide mixture (65% N_2O + 25% Ar + 5% O_2 + 5% CO_2) produced ethylene with a maximum peak (25 μmol/kg) at 8 days (Fig. 1B), which was lower than the ethylene production found for slices packed in air, but higher than those found for conventional MAPs (Fig. 1A). In contrast, the Ar and CO_2 mixture (80% Ar + 20% CO_2) was able to completely inhibit ethylene accumulation for the whole cold storage period. Moreover, Cortellino *et al.* (2015) results highlighted that the argon and nitrous oxide mixture (65% N_2O + 25% Ar + 5% O_2 + 5% CO_2) was less effective in inhibiting ethylene production than the conventional mixture (90% N_2 + 5% O_2 + 5% CO_2), even though both atmospheres were characterized by the same percentage of O_2 (5%). This finding disagrees with Leshem and Wills (1998) results suggesting that N_2O is a potent antagonist of ethylene production as well of its action. Furthermore the anti-ethylene effects of a continuous 80% N_2O + 20% O_2 gas treatment in the ripening and senescence sequences in two typical climacteric fruit, tomato and avocado, was demonstrated for the first time by Gouble, Fath, and Soudain (1995). In preclimateric treated fruit, nitrous oxide largely extended the lag period, and additively lowered ethylene production rate in tomatoes. In fruits treated at the climacteric stage, nitrous oxide markedly inhibited autocatalytic ethylene evolution. Little is known about the mechanism of action of N_2O on plants. Nitrous oxide is known to bind to lipids and also to proteins, such as cytochrome *c* oxidase (Gouble *et al.*, 1995). Sowa and Towill (1991) reported the action of N_2O on partial and reversible inhibition of respiration and cytochrome *c* activity in mitochondrial particles isolated from seed, leaf or cellular suspensions.

Firmness

Many quality degradations occurs during processing and storage of fruit, but softening is one of the most undesirable effects. Consumer has an expectation that processing and storing a product will not interfere with the anticipated sensory properties. So limiting the textural changes in minimally processed apples is highly important in order to get the consumer acceptance of the product. Firmness is determined largely by the physical anatomy of the tissue (cell size, shape and packing, cell wall thickness and strength), and the extent of cell-to-cell adhesion, together with turgor status (Toivonen & Brummel, 2008). The severity of wounding (peeling, coring and slicing) can be greater for climacteric fruit for which wound-induced ethylene promotes ripening and softening. Another factor involved in the decline of desirable texture is water loss, which leads to a decrease in turgor and crispness. It is rapid in fresh-cut product due to the absence of a cuticle and sub-epidermal layers and, hence, to the exposure of internal tissue. This degenerative phenomenon can be greatly delayed by appropriate packaging (Toivonen & Brummel, 2008). Physical and chemical changes may affect textural integrity, but also the enzymatic hydrolysis of cell wall pectic substances, due to mechanical stress of plant tissues, resulting in a loss of firmness (Varoquaux, Lecendre, & Varoquaux, 1990). Specifically, softening may also be caused by the hydrolysis of protopectins to water soluble pectins, the decrease in cellulose crystallinity, thinning of cell walls, diffusion of sugar to the intercellular spaces, ion movement from the cell wall (Toivonen & Brummel, 2008). Therefore it is difficult to preserve the texture of fresh-cut apples. Varoquaux and Wiley (1997) reported that modified atmospheres may limit the loss of compartmentalization within cells and the interaction of enzymes, such as polygalacturonases and pectin esterases, with their substrates. Many studies have demonstrated that the decrease of apple firmness during storage time is strongly dependent on the availability of oxygen, given mainly by packaging atmosphere but also by permeability of the package plastic material. The research group of Soliva-Fortuny tested the packaging in 100% N_2 combined with bags of low oxygen permeability (15 cm^3 O_2 m^{-2} bar^{-1} day^{-1}), with effective results in preserving apple softening till 21 (Soliva-Fortuny *et al.*, 2005) and 60 (Soliva-Fortuny, Lluch, Quiles, Grigelmo-Miguel, & Martín-Belloso, 2003b; Soliva-Fortuny *et al.*, 2002) days of storage. An interesting evaluation of microstructural modification by Cryo–Scanning Electron Microscopy (Soliva-Fortuny *et al.*, 2003b) identified the formation of a great quantity of exudates, as droplet-shape on the external surface of the cell walls, in fresh-cut apples stored under 2.5% O_2 + 7% CO_2 atmosphere, while sample stored under 100% N_2 showed an

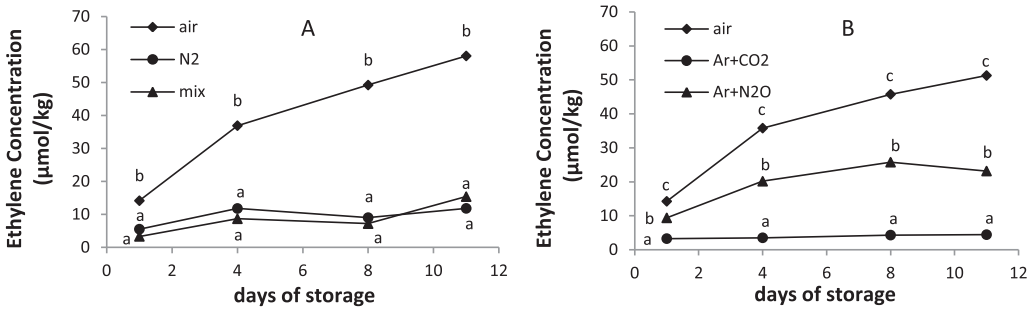

Fig. 1. Ethylene concentration in packages of dipped apple slices packed in conventional (A) and alternative (B) modified atmosphere: air, N_2 (99% N_2 + 1% O_2), mix (90% N_2 + 5% O_2 + 5% CO_2), Ar + N_2O (65% N_2O + 25% Ar + 5% CO_2 + 5% O_2), Ar + CO_2 (80% Ar + 20% CO_2). Different letters ($P < 0.05$) correspond to a significant difference among the atmospheres at the same storage time (Cortellino *et al.*, 2015).

intermediate state of deterioration between fresh apple and those packed in 2.5% O_2 + 7% CO_2 gas mixture, with droplets formation on cell surface, but also smooth and more integer cell walls. The reason of this behavior may be the maintenance of an aerobic metabolism, as long as O_2 is available in the tissue, that causes cell damages. Contrasting results were obtained by Rojas-Graü *et al.* (2007), who found that firmness of apple slices was not significantly affected either by the composition of the packaging atmosphere (air and 2.5% O_2 + 7% CO_2) or by the dipping treatment, but it was greatly influenced by the type of antibrowning agent used. In fact apple slices processed from ripe fruit and dipped in ascorbic acid solution presented lower values of firmness than slices treated with N-acetylcysteine, independently of packaging atmosphere, even though this phenomenon was not observed for samples prepared from mature-green and partially ripe fruit. This effect was reported also by Ponting, Jackson, and Watters (1972), who demonstrated that acid solutions containing ascorbic acid significantly reduced apple slice firmness.

Few research data are available about the preserving action on firmness of non-conventional gases (Ar and N_2O). Rocculi *et al.* (2004) found that firmness increased in all samples of 'Golden Delicious' apple slices packed both in air and in MAP, and suggested that this result may be explained by the effect of $CaCl_2$ used in the dipping solution, as confirmed in other experiments (Abbott, Conway, & Sams, 1989; Glenn & Poovaiah, 1990). However, apple slices packed in modified atmospheres composed of 5% O_2, 5% CO_2 together with N_2O and/or Ar (the remaining 90%) showed the greatest firmness increase throughout 12 days of cold storage. On the other hand, Cocci *et al.* (2006), testing the mixture 90% N_2O + 5% O_2 + 5% CO_2, obtained conflicting results. In fact, apple slices without dipping treatment packaged in MAP (sample MA) showed the highest firmness values for all the storage period, while those packaged in air (control) had slightly but not significantly decreasing firmness at the end of storage. In contrast, dipped apple slices showed about a 50% firmness decrease (from 8.50 to 4.50 N) just after 1 day of cold storage, maintaining these levels until the end of the experiments, independently of the MA used. This finding confirmed that the dipping treatment in ascorbic and citric acids solution caused a structural breakdown of the fruit, with consequent softening of apple tissue, as also confirmed by other researchers (Gil *et al.*,1998; Ponting *et al.*, 1972; Rojas-Graü *et al.*, 2007). Furthermore, Pardilla, Mor-Mur, Vega, and Guri (2015) verified that the firmness loss of 'Golden Delicious' apple slices, treated with 35% CO_2 + 5% O_2 + 60% N_2 atmosphere was statistically lower than in slices packaged in 90% Ar + 5% CO_2 + 5% O_2 and 90% N_2 + 5% CO_2 + 5% O_2 atmospheres between days 0 and 4; however, the presence of argon did not improve firmness property respect to the regular gas mixture.

On the other hand Cortellino *et al.* (2015) results concerning firmness of undipped 'Golden Delicious' apple slices showed that the conventional mixtures 99% N_2 + 1% O_2 and 90% N_2 + 5% O_2 + 5% CO_2 (Fig. 2) preserved better this important quality parameter than air atmosphere, even though the difference was not always statistically significant. If the other atmospheres studied by Cortellino *et al.* (2015) are considered, the Ar + CO_2 combination (80% Ar + 20% CO_2) (Fig. 2) positively and significantly influenced firmness of apple slices during the whole shelf life. In contrast, the Ar + N_2O mix (65% N_2O + 25% Ar + 5% O_2 + 5% CO_2) had not any influence on slice firmness, as Ar + N_2O packaged slices were as firm as those packed in air. Furthermore, Cortellino *et al.* (2015) found that the beneficial effect of modified atmosphere on slice firmness was completely nullified by the dipping treatment for the conventional mixes and only partially for the Ar + CO_2 combination, whose samples were characterized by somewhat higher, even if not significant, firmness values. This neutralizing effect of dipping treatment with anti-browning agents (ascorbic and citric acids) on MAP preservation tissue apple from softening was in agreement with Cocci *et al.* (2006) and Rojas-Graü *et al.* (2007) findings. Even though the ethylene production of undipped slices might have been slightly different compared with that of dipped apples, as found by Gil *et al.* (1998) and Rojas-Graü *et al.* (2007), the results of undipped apple firmness confirmed the relationship between ethylene production and firmness retention during the shelf life.

Browning

The appearance of a fresh-cut fruit is the attribute with the most immediate impact on the consumer and strongly affects the decision to buy (Toivonen & Brummel, 2008). This represents a problem especially for fruit with a white flesh such as apple and pear. The oxidative browning process is triggered by the breakdown of membranes inside cells of plant tissues (Toivonen, 2004) and by the resulting mixing of polyphenol substrates with polyphenol oxidase (PPO). In the presence of oxygen, the enzyme PPO catalyzes two reactions: (1) hydroxylation of monophenols to diphenols and (2) oxidation of diphenols to quinones. The hydroxylation reaction is relatively slow and results in colorless products, while the oxidation reaction is relatively rapid and the resultant quinones are colored. Subsequent reactions of the quinones lead to melanin accumulation, which is the brown or black pigment associated to "browning" in plant tissues. The specific reaction sequence which results in brown or black-colored products depends on the specific structure of the polyphenolic substrate (Toivonen & Brummel, 2008). Tissue browning is mostly due to changes in lightness (L^*) and in the greenness-redness (a^*) of the pulp and may be caused by the enzymatic action of catechol oxidases, which are the most common polyphenol oxidases in apple fruit (Harel, Mayer, & Shain, 1964). The most important factors that determine the rate of enzymatic browning in fruit and vegetables are the concentrations of both active PPO and phenolic compounds, the pH, the temperature and the oxygen availability of the tissue (Martinez & Whitaker, 1995). As oxygen is required by PPO at the site of wounding to initiate the browning reaction, the exclusion of O_2 is used in juices and wines, by bottling them under nitrogen, in order to prevent the onset of this degrading phenomenon (Martinez & Whitaker, 1995). To this aim the use of O_2-impermeable packaging or edible films may also be useful. Because the O_2 is needed for browning reactions, MAP with low O_2 and high CO_2 levels can positively contribute to avoid browning in fresh-cut products. However, low O_2 and elevated CO_2 atmospheres can not effectively inhibit browning of fresh-cut fruit and vegetables such as apple, banana, pear, potato or artichoke, because of their high phenolic content (Rojas-Graü, Oms-Oliu, Soliva-Fortuny, & Martín-Belloso, 2009). Consequently MAP system has to be combined with antioxidant treatments to delay browning of fresh-cut tissues.

Soliva-Fortuny *et al.* (2002) for 'Golden Delicious' apple slices reported a limited action of the depletion of L^* values throughout storage (60 days) by the packaging under 100% N_2 in comparison with air, independently of dipping treatment. The same author (Soliva-Fortuny, Grigelmo-Miguel, Odriozola-Serrano, Gorinstein, & Martín-Belloso, 2001) proved that N_2 combined with the use of packaging with low oxygen permeability was more efficient than 2.5% O_2 + 7% CO_2 atmosphere in limiting color difference (ΔE) and the change of L^* value. However, Soliva-Fortuny *et al.* (2001) described also an intense reduction of enzymatic activity (PPO) by the presence of CO_2 (2.5% O_2 + 7% CO_2 + 90.5% N_2) compared with nitrogen (100% N_2) in the packaging atmosphere. Similar results were previously observed for not treated sliced apples packed in presence of CO_2/N_2 (20:80 v/v) (Nicoli, Anese, & Severino, 1994) and N_2 (Anese *et al.*, 1997) atmospheres: in both the experiments a

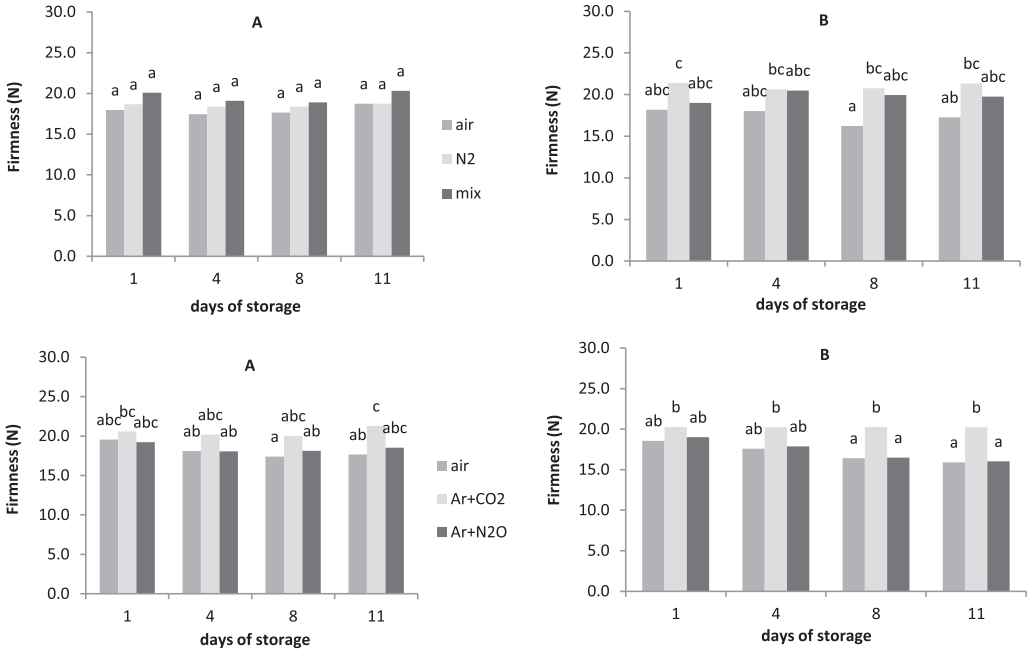

Fig. 2. Firmness (N) of fresh-cut apples dipped (A) and no-dipped (B) before packing in air, in conventional N_2 (99% N_2 + 1% O_2), mix (90% N_2 + 5% O_2 + 5% CO_2) and alternative modified atmospheres of Ar + N_2O (65% N_2O + 25% Ar + 5% CO_2 + 5% O_2) or Ar + CO_2 (80% Ar + 20% CO_2). Different letters ($P < 0.05$) correspond to a significant difference (Cortellino *et al.*, 2015).

limited decrease of hue angle coupled to a limited increase of lightness difference between the final and the initial L* values (ΔL*) were found. Rojas-Graü *et al.* (2007) proved that browning intensity of apples dipped in N-acetylcysteine solution, evaluated by a decrease in L* value, was much higher in samples packaged under air than those packed under 2.5% O_2 + 7% CO_2 atmosphere, whereas apple slices dipped in ascorbic acid, showing lower initial L* values (about 72) than samples treated with N-acetylcysteine (about 76), behaved independently of packaging atmosphere.

Rocculi *et al.* (2004) tested the efficiency of alternative (Ar and N_2O) modified atmospheres for the inhibition of browning phenomenon obtaining positive results. In fact apple slices packed in 90% N_2O + 5% CO_2 + 5% O_2 and in 65% N_2O + 25% Ar + 5% CO_2 + 5% O_2 atmospheres showed higher values of whiteness index and hue angle as well as lower Chroma (less saturated color) than apple slices packed in air and in the conventional mix (90% N_2 + 5% CO_2 + 5% O_2). These results were also confirmed by image analysis, as samples packed in N_2O–Ar mixture showed the lowest browning level (15% of browning area) and those packed in N_2O–CO_2–O_2 mixture an intermediate level (about 25%) in comparison with air (about 70%) and conventional mix (about 80%). The authors hypothesized that these results could be due to the higher solubility of Ar respect to N_2 and that Ar is in competition with O_2 at chemical-enzymatic level; in fact argon, having the same solubility and molecular weight as O_2, replaces it causing PPO inhibition (Day, 1996). Moreover Spencer's (1995) experiments indicated that noble gases are biochemically active, probably due to their higher solubility in water compared with nitrogen and to the possible interference with enzymatic oxygen receptor sites. In Rocculi *et al.* (2004) experiment these effects could be enhanced due to the low packaging permeability, that allowed the retention of MA inside the boxes, as well as to the interaction of MA with the product. In another experiment carried out by Cocci *et al.* (2006), it was proved that the N_2O mixture (90% N_2O + 5% CO_2 + 5% O_2) was efficient in limiting color change provided that its use was combined with anti-browning dipping (ascorbic and citric acids). In fact there was not any significant difference in the level of percent browning area between not dipped samples packed in air and in the N_2O mixture, showing the highest values (around 90%) just after the first day of storage, and maintaining high levels of percent browning area until the end of the experiment (from about 70 to 85%). On the other hand, if dipping was applied, sample packed in modified atmosphere showed a significant lower browning level (ranging from 10 to 20%) over all the storage period than those packed in air, whose browning area ranged from about 25% just after 1 day to about 50% after 6 days of cold storage. Concerning the influence of argon on polyphenol oxidase (PPO), a specific study on mushroom and apple conducted by O'Beirne, Murphy, and Ni Eidhin (2011) demonstrated that apple and mushroom PPO were more inhibited by atmospheres enriched with argon than by those enriched with nitrogen: at each concentration of argon, the K_m value (constant of Michaelis–Menten) of mushroom and apple PPO was greater than that for the corresponding level of nitrogen. Atmospheres containing low level of O_2 (air enriched with nitrogen) are known to inhibit PPO reducing the availability of oxygen through dilution (Ballantyne, Stark, & Selman, 1988; O'Beirne, 1990). As an inhibitory effect on PPO activity was still recorded when the oxygen was present at a maximum concentration (21%) and the balance was argon instead of nitrogen, such effect is characteristic of competitive inhibition (O'Beirne *et al.*, 2011). Since the inhibitory effects of argon were greater than those of nitrogen, inhibition of argon must be greater than the dilution effect of nitrogen. Previously Spencer, Schvester, and Boisrobert (1998), in their patent, attributed the special "inhibitory" effect of argon and other noble gases on PPO activity in low-O_2 atmospheres in a nonspecific way to molecular effects such as polarizability, iconicity, Van der Waals force and atomic radii. However Spencer *et al.* (1998) demonstrated that the greater inhibitory action of argon and other noble gases, compared with nitrogen, was linked to the atomic mass: the higher the atomic mass the greater the inhibition. The findings of O'Beirne *et al.* (2011) are comparable with the theory that the "additional benefits" of argon are directly related to the higher Van der Waals radius value

of argon compared with nitrogen, being 1.91 Å for argon and 1.54 Å for nitrogen. O'Beirne *et al.* (2011) found that the inhibitory effects of argon on apple PPO were 1.2/1.5-times than that of nitrogen with atmospheres characterized by different oxygen contents (3–21%). Furthermore, the results by Zhang, Quantick, Grigor, Wiktorowicz, and Irven (2001) indicated that both nitrogen and argon reduced markedly the tyrosinase (PPO) activity and the reaction of the enzyme with its substrate, compared to those without gas treatment. In details tyrosinase activity with argon treatment was reduced by up to 14.2% more than nitrogen treatment when treated directly, and by up to 22.6% in the mixture of the enzyme and substrate. This may be because argon has a better ability than nitrogen to reduce the level of dissolved oxygen whose presence is necessary for tyrosinase to catalyze the reaction. Argon is reported to be biochemically active, probably due to its enhanced solubility in water compared with nitrogen (Spencer, 1995).

In this context the study conducted by Cortellino *et al.* (2015) (Fig. 3) on 'Golden Delicious' apple slices concerned the evaluation of conventional and modified atmosphere, combined or not with dipping treatment (citric and ascorbic acid), in preventing browning. The technological step of dipping caused a decrease of the browning index (BI) from 28 to 26, indicating that a slight phenomenon of pulp whitening occurred with the dipping treatment. The BI value of dipped samples packed in air increased during the shelf life even though they reached only the initial value of fresh slices (Fig. 3A). Both conventional modified atmospheres (99% N_2 + 1% O_2 and 90% N_2 + 5% O_2 + 5% CO_2) and the Ar + CO_2 combination (80% Ar + 20% CO_2) successfully maintained the initial value of BI for all the 11 days of cold storage. In contrast, the slices packed in Ar + N_2O mix (65% N_2O + 25% Ar + 5% CO_2 + 5% O_2) showed the same behavior as those stored in air. In slices without dipping treatment, browning developed quite quickly in the first 24 h; then, slices packaged in air showed a high value of BI (~40), which remained stable or decreased slightly with cold storage time. The two conventional MAP atmospheres induced more degrading phenomenon (higher BI) till the fourth day of storage than air atmosphere. Afterward, the BI of slices packed under conventional MAPs decreased and reached the value of sample packed in air, and this fact may be due to further biochemical processes leading to the formation of less dark color compounds. The same initial phenomenon was also observed for slices packed in alternative MAP, then the Ar + N_2O sample behaved as air sample and the Ar + CO_2 sample showed the worst performance with higher BI value. On the whole, data proved that MAP without or with low level of oxygen could not effectively prevent browning in absence of a dipping treatment. Specifically argon played a contradictory role as the atmosphere with 80% Ar controlled the browning in dipped sample but enhanced it in undipped ones, in agreement with the conflicting results by Day (1996) but in disagreement with Spencer's (1995) findings.

The effectiveness of superoxygen atmospheres in preserving color characteristics was little known. A recent study by Ghidelli *et al.* (2012) indicated that the use of soy protein-based coatings in combination with an elevated O_2 (80 kPa O_2) atmosphere allowed to retain slightly lower a* value of apple pieces than when packed in air and in 15 kPa CO_2 + 5 kPa O_2 atmosphere, but the subjective visual quality ratings did not differ among the atmosphere treatments. In addition, Day (2001) reported that high O_2 and high Ar MAP did not prevent the enzymic browning of non-sulphite dipped apple slices, even if no further browning took place after pack opening.

Fermentative metabolites

Modified atmosphere packaging systems can severely modify the fruit volatile profile (Rojas-Graü *et al.*, 2009). Ke, Goldstein, Omahony, and Kader (1991) reported that ethanol and acetaldehyde are the main products of fermentative metabolism in fruits and their accumulation is correlated with off-flavor development. Lakakul, Beaudry, and Hernandez (1999) pointed out the importance of maintaining in the package headspace both O_2 levels just above the fermentation threshold and CO_2 concentrations below the range that causes injury. Thus, under specific circumstances, low O_2 and high CO_2 may lead to the production of fermentative

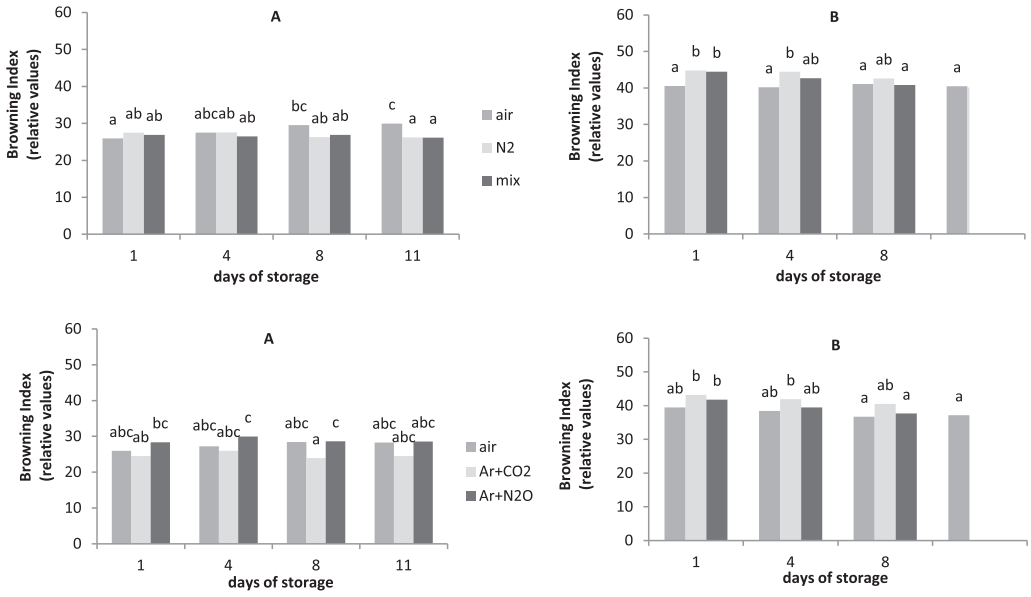

Fig. 3. Color (Browning Index) of fresh-cut apples dipped (A) and no-dipped (B) before packing in air, in conventional N_2 (99% N_2 + 1% O_2), mix (90% N_2 + 5% O_2 + 5% CO_2) and alternative modified atmospheres of Ar + N_2O (65% N_2O + 25% Ar + 5% CO_2 + 5% O_2) or Ar + CO_2 (80% Ar + 20% CO_2). Different letters ($P < 0.05$) correspond to a significant difference (Cortellino *et al.*, 2015).

metabolites, which are responsible for unpleasant off-flavors and odors. Too low O_2 atmospheres may trigger anaerobic metabolism in fresh-cut fruit resulting in an increase of fermentation. It has been shown that the maximum rate of O_2 uptake increases with increasing temperature, and that the lowest O_2 partial pressure to which fruit could be exposed without having the onset of fermentation processes also increases with increasing temperature (Lakakul *et al.*, 1999). Besides, it has been suggested that CO_2 dissolution enhances acidity in the cell medium and may be responsible for physiological disorders. High CO_2 concentrations also inhibit several enzymes of Krebs' cycle, including succinate dehydrogenase, which either triggers anaerobic respiration or results in the accumulation of succinic acid, which is potentially toxic to the fruit tissue (Varoquaux, 1991). The use of high O_2 atmospheres have been also proposed to prevent anaerobic conditions and to reduce the production of fermentative metabolites (Day, 1996). However the stress response, caused by the highly oxidative environment combined with the accumulation of carbon dioxide into the packages, induces ethanol accumulation throughout storage in fresh-cut melons and pears packaged under 70 kPa O_2 (Oms-Oliu, Raybaudi-Massilia Martinez, Soliva-Fortuny, & Martín-Belloso, 2008; Oms-Oliu, Soliva-Fortuny, & Martín-Belloso, 2008). No literature data are available about the content of fermentative metabolites in apple slices packed under superoxygen atmosphere. The most accepted explanation for O_2 toxicity is the formation of superoxide radicals (O_2^-), which are destructive to cell metabolism (Fridovitch, 1975).

Soliva-Fortuny *et al.* (2002) and Rojas-Graü et al. (2007) studied the evolution of the headspace ethanol developed by apple slices packaged under air and modified atmospheres (100% N_2 and 2.5% O_2 + 7% CO_2 + 90.5% N_2). Soliva-Fortuny *et al.* (2002) observed a mild but progressive accumulation of ethanol during the first 40 days of storage with similar levels under all packaging conditions (air and 100% N_2). Then, an important increase was triggered in bags under air, while only a slight rise in ethanol levels was observed in 100% N_2 and 2.5% O_2 + 7% CO_2 + 90.5% N_2 packages. Under air, ethanol production was avoided during the first 10 days of storage, when there was a high availability of O_2 in the package headspace which prevented apple tissue from anaerobic metabolism. However, ethanol concentrations rose markedly beyond 6 weeks of storage, showing a sudden increase of anoxic pathways that may be due to the more severe conditions underwent by apple slices.

Rojas-Graü *et al.* (2007) compared the influence of air and 2.5% O_2 + 7% CO_2 + 90.5% N_2 atmosphere on the headspace of 'Fuji' apple slices packages. It was highlighted a slight but progressive accumulation of ethanol until 2 weeks of storage. The successive increase in the headspace ethanol levels was regardless of the type of packaging atmosphere and of dipping treatment, but it depended on maturity degree of fruit; in fact a sudden ethanol increase was triggered only in ripe apple slice samples after 3 weeks of storage, but not in samples prepared from partially-ripe or mature-green fruit. This rise could have been triggered by the low O_2 concentrations inside of packages observed in samples prepared from ripe fruit after the third week of storage.

A different approach was carried out by Cortellino, Gobbi, and Rizzolo (2014a), who didn't evaluate the fermentative metabolite composition in the packages' headspace, but that of apple slice tissue, considering not only ethanol but also acetaldehyde and ethyl acetate (Fig. 4). The content of ethanol increased progressively starting from the first day of storage for all MAP treatments, but only after 8 days of cold storage for slices packaged under air. Samples packaged under 80% Ar + 20% CO_2 atmosphere showed the highest production of ethanol throughout the whole storage time. At the end of shelf-life (11 days) apples under 80% Ar + 20% CO_2 and

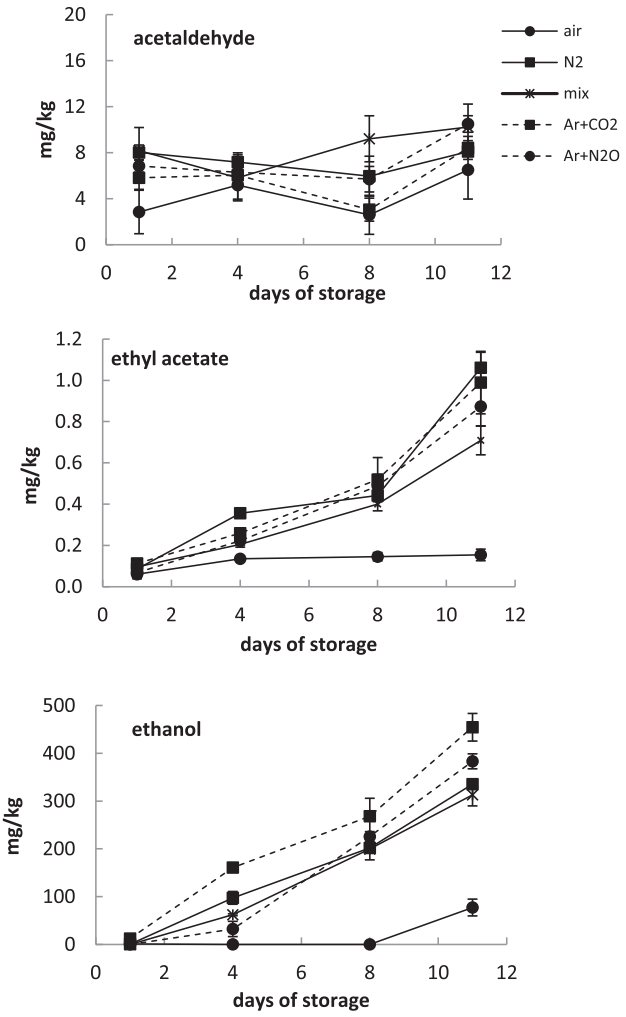

Fig. 4. Content of acetaldehyde, ethanol and ethyl acetate of fresh-cut apples packed in air, in conventional N_2 (99% N_2 + 1% O_2), mix (90% N_2 + 5% O_2 + 5% CO_2) and alternative modified atmospheres of Ar + N_2O (65% N_2O + 25% Ar + 5% CO_2 + 5% O_2) or Ar + CO_2 (80% Ar + 20% CO_2). Media ± st. error (Cortellino *et al.*, 2014a).

65% N_2O + 25% Ar + 5% CO_2 + 5% O_2 alternative MAPs were characterized by higher values of ethanol content (350–450 mg kg^{-1}) than samples under 99% N_2 + 1% O_2 and 90% N_2 + 5% O_2 + 5% CO_2 conventional MAPs (~300 mg kg^{-1}), while apple slices packed under air developed limited quantity of ethanol (<100 mg kg^{-1}). The overall results underlined that the production of ethanol is greatly influenced by the packaging atmosphere, in disagreement with both Soliva-Fortuny *et al.* (2002) and Rojas-Graü *et al.* (2007) findings. Moreover, the results confirmed that the absence or the presence of low O_2 concentration leads to production of fermentative metabolites, like ethanol, which are responsible for unpleasant off-flavors and odors. Apple slices produced also acetaldehyde in the 3–10 mg kg^{-1} range, but without a clear trend. Air sample developed a somewhat lowest amount of this fermentative product. However, its production seems to be independent from the packaging atmosphere. Apple samples, except those packed under air, produced also increasing amounts of ethyl acetate from 0.1 mg kg^{-1} at the first day of cold storage for all the samples to 0.6–1.1 mg kg^{-1} at the end of storage time for all the MAP samples: slices packed either under only 1% O_2 (99% N_2 + 1% O_2) or completely anoxic (80% Ar + 20% CO_2) atmospheres produced higher quantities of ethyl acetate than the other two packaging

conditions characterized by 5% O_2 (90% N_2 + 5% O_2 + 5% CO_2 and 65% N_2O + 25% Ar + 5% CO_2 + 5% O_2). On the other hand, apple slices packaged under air developed very low amounts (~0.15 mg kg^{-1}) of ethyl acetate during the whole cold storage time. On the whole the considerations regarding the correlation between packaging condition and ethyl acetate development are very similar to those previously reported for ethanol.

The physiological respiratory activity data involves CO_2 production and O_2 consumption (Fonseca, Oliveira, & Brecht, 2002). Aerobic respiration consists of oxidative breakdown of organic reserves to simpler molecules, including CO_2 and water, with release of energy. The process consumes O_2 in a series of enzymatic reactions. Glycolysis, the tricarboxilic acid cycle, and the electron transport system are the metabolic pathways of aerobic respiration. The respiratory quotient (RQ), which is the ratio between CO_2 production and O_2 uptake, is normally assumed to be equal to 1.0 if the metabolic substrates are carbohydrates. When this value falls in the range of 0.7–1.3, it denotes that a product aerobically respires (Makino, 2013). The RQ is much greater than 1.0 when anaerobic respiration takes place. In fermentative metabolism, ethanol production involves decarboxylation of pyruvate to CO_2 without O_2 uptake (Fonseca *et al.*, 2002). Concerning this point of view Cortellino, Gobbi, and Rizzolo (2014) confirmed that for samples packed under either nitrogen (99% N_2 + 1% O_2) or low oxygen (90% N_2 + 5% O_2 + 5% CO_2) atmosphere a fermentative anaerobic respiratory process occurs, since high respiratory quotient values (RQ>>1) were found from the beginning of cold storage This phenomenon didn't occur for apples packed under air (RQ < 1), which showed an RQ = 1.22 only at the end of shelf life indicating that fermentative metabolism was just triggered only when fruit slices were near to the senescence. Furthermore Soliva-Fortuny *et al.* (2005) observed a noticeable rise in RQ during the third week of storage, especially in fresh-cut apples packaged under initial 0 kPa O_2 atmosphere, suggesting that fermentative anaerobic respiratory processes had begun to be triggered. The increase in RQ coupled with declining O_2 partial pressure determines the fermentation threshold, which in apple slices was found to be about half of the O_2 partial pressure for whole apples (Lakakul *et al.*, 1999). O_2 partial pressures can be lower for cut fruits than for whole apples because skin removal reduces the diffusion path length, thus increasing gas permeation to the surrounding environment (Soliva-Fortuny *et al.*, 2005).

The changes in aroma fingerprint of fresh-cut apples during the shelf life period can be detected with a fast and simple approach by electronic nose system, a sensor-based technology, which responds to the whole set of headspace volatiles, creating a unique digital pattern. Usually, a little portion of fruit tissue (3–10 g) is sealed in a vial and, after an equilibration time, the headspace gas is sampled by an automatic sampler and injected into the sensor chamber. This methodology was used by Tanprasert, Beaudry, and Harte (2007) to study the influence of postharvest treatments on 'Jonagold' apple slices packed under air, by Guarrasi, Giacomazza, Germanà, Amenta, and San Biagio (2014) to study the influence of a traditional MAP on 'Fuji' apple slices, and by Siroli *et al.* (2014) to study the use of various natural antimicrobials to extend the shelf life of 'Golden Delicious' apple slices packed under a traditional MAP. Tanprasert *et al.* (2007) reported that there is an influence of postharvest treatments of fruit before processing (storage atmosphere, time of storage, 1-methylcyclopropene treatment) on electronic nose pattern of 'Jonagold' apple slices packed under air only at the beginning of the shelf life period at 3 °C; after 2 weeks of shelf life the effect of processing overrides the effect of postharvest treatments. Guarrasi *et al.* (2014) found a different e-nose pattern between 'Fuji' apple slices packed in air and under 100% N_2, and, for both the treatments, changes in e-nose pattern during the 14 days of shelf-life at 4 °C. Siroli *et al.* (2014) showed that the addition to the dipping solution of natural antimicrobials, such as hexanal, citral, 2-(*E*)-hexenal, citron EO and carvacrol, alone or in combination, combined with MAP (7% O_2, 0% CO_2) quite unaffected the e-nose pattern, which indeed changed during the 35 days of shelf life at 6 °C. A different approach was carried out by Cortellino, Rizzolo, *et al.* (2014), who performed the electronic nose analyses with the commercial portable PEN3 (Win Muster Airsense Analytic Inc.) on the packages, before opening them for the analysis of apple slices. They compared the electronic nose patterns of 'Golden Delicious' apple slices packaged under air and 99% N_2 + 1% O_2 and 90% N_2 + 5% CO_2 + 5% O_2 modified atmospheres, processed at harvest and after 7 months' storage at 2 °C in a controlled atmosphere (1% O_2 + 2% CO_2). Results highlighted that the responses of W5S, W1S and W2S sensors were higher for samples from stored fruit than those from fruit processed at harvest independently from the type of the packaging atmosphere, and increased during the 10 days of shelf life at 4 °C; in contrast, the responses of W1C and W5C sensors gradually decreased throughtout the shelf life and in a similar way in all the treatments. By coupling the electronic nose data with discriminant analysis, Cortellino, Rizzolo, *et al.* (2014) were able to distinguish for every packaging atmosphere the samples processed at harvest from those processed after storage during the whole shelf life time, obtaining 93.55% (air), 100% (99% N_2 + 1% O_2) and 96.77% (90% N_2 + 5% CO_2 + 5% O_2) of correct classification percentages for the combination of processing time and days of shelf life.

Sensory characteristics

Very little sensory studies have been carried out to evaluate the influence of packaging conditions on the quality perception of fresh-cut apples by trained judges. In fact most of the research evaluated instrumentally the visual and texture quality, as well as the off-flavors presence. An interesting approach was realized by Soliva-Fortuny *et al.* (2005), where 'Golden Delicious' apple cubes treated with calcium chloride and packaged under modified atmospheres were compared with those prepared just before consumption. Panelists gave low visual quality scores only to sample preserved under an initial packaging atmosphere of 2.5 kPa O_2 + 7 kPa CO_2 in plastic packages of lower permeability, at 3 weeks of storage, coinciding with the greatest color variation. No other differences were detected between treatments. For panelists all treated samples had slightly higher or not significantly different scores for firmness than untreated freshly prepared apple cubes. The reason may be due to the great protective effect of calcium chloride treatments that hindered the packaging atmosphere influence. These observations are supported by Soliva-Fortuny *et al.* (2002) results showing that calcium treatments have much more influence than MAP on the texture preservation of fresh-cut apples. Acidity scores also didn't have significant changes, whereas sweetness scores decreased during the second and third week of storage, suggesting a reduction in the amount of sugars. Acceptance scores for the overall quality of apple cubes dropped gradually throughout storage. At 3 weeks of storage these scores fell to low values as the off-odors presence started to be detectable by panelists. This trend was emphasized in samples packaged in 2.5 kPa O_2 + 7 kPa CO_2, which showed a faster quality degradation, suggesting that high CO_2 concentrations were more detrimental to the sensory quality than storage under anoxic conditions.

Considering this scarce background, sensory analysis carried out by Cortellino *et al.* (2015) on 'Golden Delicious' apple slices packed in air, conventional MAPs (99% N_2 + 1% O_2 and 90% N_2 + 5% O_2 + 5% CO_2) and alternative MAPs (80% Ar + 20% CO_2 and 65% N_2O + 25% Ar + 5% CO_2 + 5% O_2) provides useful information (Fig. 5).

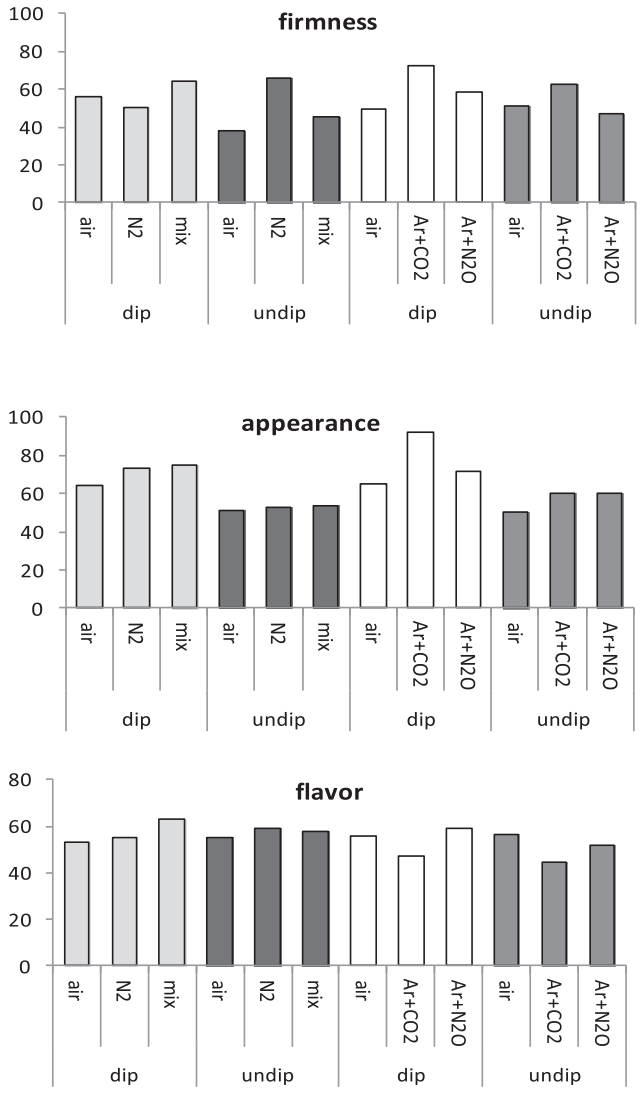

Fig. 5. Sensory evaluation of firmness, appearance and flavor of fresh-cut apple slices after 11 days of refrigerated storage, dipped and no-dipped before packing in air, in conventional N_2 (99% N_2 + 1% O_2), mix (90% N_2 + 5% O_2 + 5% CO_2) and alternative modified atmospheres Ar + N_2O (65% N_2O + 25% Ar + 5% CO_2 + 5% O_2) or Ar + CO_2 (80% Ar + 20% CO_2) (Cortellino *et al.*, 2015).

Considering that the exposure to O_2 and CO_2 levels outside the limits of tolerance led to anaerobic respiration with the production of undesirable metabolites and other physiological disorders, as found by Soliva-Fortuny *et al.* (2002), Cortellino *et al.* (2014a), Cortellino, Rizzolo, *et al.* (2014), it was important to consider the flavor judgment. The concentration of fermentation product did not negatively influence the flavor evaluation of panelist in the case of conventional MAPs and Ar + N_2O mixture after 11 days of storage, while this sensory attribute was slightly affected and only after 8 days of shelf-life, for the Ar + CO_2 combination (80% Ar + 20% CO_2). The sensory tests partially confirmed the instrumental results of firmness. Concerning this sensorial aspect, the no-dipped slices packed in nitrogen and Ar + CO_2 atmospheres, as well as the dipped (in anti-browning solution) sample packed in Ar + CO_2, obtained higher scores. The appearance attribute score was extremely dependent on the application of dipping pre-treatment: in fact if the anti-browning treatment was not applied, low scores (~50) for appearance were given independently of the atmosphere composition. Furthermore, in order to study in depth the interaction between alternative MAPs and anti-browning dipping treatment on sensory profile of 'Golden Delicious' apple slices packed in air, 80% Ar + 20% CO_2 and 65% N_2O + 25% Ar + 5% CO_2 + 5% O_2 during shelf life at 4 °C, Cortellino, Gobbi, and Rizzolo (2014b) analysed the sensory scores related to the intensity of firmness and crispness and to the pleasantness of appearance and flavor by Cluster analysis, using Ward's method and Square Euclidean distance metric. In this way samples were grouped into five clusters, each one having a distinctive sensory profile. Dipped slices packaged in Ar + CO_2 and Ar + N_2O mixtures after 1 day of shelf life at 4 °C were judged the firmest (75.2) and the most crispy (68.8), with the highest pleasantness for flavor (67.8) and appearance (89.6); the undipped slices from all the 3 atm after 1 day of shelf life had medium–high values for firmness (69), crispness (60.2) and flavor pleasantness (62); the dipped and undipped slices packed in air and the undipped ones packed in Ar + N_2O mixtures after 4, 8 and 11 days of shelf life at 4 °C were the least firm (49.6) and crispy (38.7), but had medium scores for pleasantness of appearance (64.6) and flavor (61.1). The dipped slices packed in Ar + CO_2 and Ar + N_2O mixtures in shelf life for more than 8 days had a good appearance (83.1), medium-low values for firmness (57.4) and crispness (52.6) and the least flavor pleasantness (43.3), due to the production of undesirable metabolites induced by the anaerobic respiration (Cortellino, Rizzolo, *et al.*, 2014; Cortellino *et al.*, 2014a, Soliva-Fortuny *et al.*, 2002), whereas those in shelf life for less than 8 days and the undipped ones packed in Ar + CO_2 obtained medium–high scores for firmness (60.2), crispness (62.7), and pleasantness of appearance (69.4) and flavor (52.3).

Conclusion

The packaging under conventional modified atmospheres, characterized by low O_2 level (1 and 5%), successfully preserved the firmness of apple slices during all refrigerated storage limiting the ethylene production. In addition also the alternative mix Ar + CO_2 was able to control the ethylene production and consequently to maintain firmness, even if an interaction between anti-browning dipping treatment and modified atmosphere was found: the preserving efficacy of MAP resulted almost completely nullified by the dipping treatment, which, based on ascorbic and citric acid, caused a structural breakdown. However, MAPs were not able to control the enzymatic browning if not combined with an anti-browning dipping treatment. The studies on the interaction between anti-browning treatments and modified atmosphere packaging highlighted the key role of sensory analysis in finding the best combination between MAP, anti-browning treatment and shelf life time. The contrasting results among the various research groups could be reasonably also due to the different periods (from 14 to 60 days) and temperatures (from 3 to 6 °C) of shelf life. Furthermore it was shown that using electronic nose it is possible to classify apple slices according to the shelf life time, and so it could be useful to manage fresh-cut apple products during production and along the distribution chain in order to limit waste.

Acknowledgment

This research was supported by Fondazioni in rete per la ricerca agroalimentare "AGER – Agroalimentare e ricerca", funding the project "STAYFRESH – Novel strategies meeting the needs of the fresh-cut vegetable sector" (N° 20102370).

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