Physical, Physiological and Microbial Deterioration of Minimally Fresh Processed Fruits and Vegetables

F. Artés*, P.A. Gómez and F. Artés-Hernández

Postharvest and Refrigeration Group, Technical University of Cartagena. Paseo Alfonso XIII 48, 30203 Cartagena, Murcia, Spain

Minimally fresh processed (MFP) or fresh-cut fruits and vegetables is currently the fastest growing subsector of the food industry with still a high potential of growth world wide. The practical advantages and convenience they provide to consumers undoubtedly favour this fact. However, because of their specific ways of preparation, MFP plant foods are highly perishable. To minimise this, they must be elaborated following strict control procedures in order to avoid quality loss, assuring food safety to consumers. The most common physical, physiological and microbial causes of deterioration that involve the preparation of these kind of products as well as the main procedures used to avoid undesirable changes are described in this review.

Key Words: fresh-cut, fruits, vegetables, mechanical damage, browning, discoloration, off-flavours, off-odours, texture, pithiness, translucency, chilling injury, modified atmosphere packaging

INTRODUCTION

Consumer's demand of minimally processed fruits and vegetables free from additives and with high overall quality and safety have experienced a sharp increment in the recent years. Fresh cut is the branch of the food industry with more noticeable rhythm and potential of growth due to its convenience, healthiness and attractive appearance and flavour. Consequently it has been highly increased the variety and quantity of fruits and vegetables prepared and conditioned for their immediate consumption (ready to eat). Methods for their elaboration include defoliating, peeling, removing parts, cutting, shredding, grating, washing, disinfecting, rinsing, draining, drying and packaging. After processing they are kept throughout distribution and retail sale under chilling and modified atmosphere packaging (MAP) which is generated by the use of polymeric films commonly provided of a selective permeability to gas diffusion. Modified atmospheres help to keep quality and extend storage life by inhibiting or lowering metabolic activity, decay, browning (Gunes and Lee, 1997), and specially the ethylene biosynthesis and action (Mathooko, 1996).

The terms used to refer to these kind of products are 'fresh-cut' (the more commonly used in USA), 'minimally or lightly processed' and 'ready to eat' (more common in Europe), 'pre-prepared', 'ready to use', and also 'fourth range' or 'IV gamme' (more common in France and Italy). Catering and collective and institutional food services represented the biggest share of the demand of these products, although it has been more recently extended to hyper and supermarkets, all kind of restaurants and food stores.

One of the main advantages of minimally fresh processed (MFP) plant foods is that they have almost the same properties than the whole intact product, with a much reduced elaboration time and with a uniform and consistent quality. They also facilitate the consumption of healthy products requiring a reduced storage place because packages contain only eatable produce. On the contrary, among the main inconveniences and concerns for consumers, it has been mentioned the variations that often take place in their quality and shelf life, the need of a strict control of low temperature and severe hygiene from elaboration to retail sale, and the requirements of raw materials with a very high quality, mainly aroma, flavour and nutritive value (Artés and Artés-Hernández, 2003).

There is an increased interest in extending the use of physical treatments, as alternatives to agro-chemicals, in order to control quality attributes losses, physiological

*To whom correspondence should be sent (e-mail: fr.artes@upct.es).
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disorders and diseases in whole and MFP fruits and vegetables. In this way, the cold chain is essential to keep the quality of these fresh products since tissues are still alive. The area used for elaboration and conditioning processes must also be completely isolated and air conditioned with a temperature under 10°C, taken into account that the lower the temperature the best the quality. It has been suggested to prepare these products in a processing room with cool clean air ('clean room' technology) with less than 10,000 particles with more than 0.5 μm diameter/m^3, and less than 70 particles with more than 5 μm diameter/m^3 (Havet and Hennequin, 1999). Furthermore, for distribution and retail sale temperatures from 0 to 1°C are recommended, although under commercial conditions 5°C should be considered as a limit (Artés and Artés-Hernández, 2003).

The attractiveness and convenience of fresh cut are helping to bring the mentioned increased consumption of fresh produce, but these benefits are offset by the rapid deterioration and short shelf life of the products and the potential health hazards associated with spoilage. Some defects are often found in MFP plant foods due to physical, chemical, microbial and enzymatic reactions that may cause their spoilage. These defects commonly result in undesirable quality attributes changes together with the development of microorganisms, like mesophilic and psycrotrofic flora, and the growth of species like *Escherichia coli*, *Aeromonas hydrophila*, *Salmonella* sp., *Clostridium botulinum*, *Listeria monocytogenes* or *Staphylococcus aureus* (that could grow below 5°C). An optimal design of the industrial installations and the use of proper techniques could minimise these negative aspects.

The attainment of a satisfactory commercial self-life starts with the election of proper raw material and continues with the execution of appropriated industrial practices of handling, processing, packaging, storage and distribution and retail sale conditions. All these factors should be complementary applied and in a synergetic way. Otherwise, the MFP products will be very susceptible to diverse alterations that adversely affect their quality and safety. Fresh cut products have a number of food quality and safety challenges that need to be addressed. Consequently, the objective of this review was to describe the main physical, physiological and microbial changes that take place during storage of minimally processed fruits and vegetables.

EFFECTS OF MECHANICAL DAMAGE

Cutting of produce removes the natural protection of the epidermis and destroys the internal compartmentation that separates enzymes from substrates. It is obvious that, after processed, plant tissues suffer physical damages that make them much more perishable than when the original product is intact. Also, the respiratory activity and ethylene emission are generally much higher, especially during the first hours after they have been cut, peeled or shredded. This phenomenon has been observed for lettuce, melon, tomato, celery, cabbage, etc. (Varoquaux and Wiley, 1994; Cantwell and Suslow, 2002; Gómez and Artés, 2005; Aguayo et al., 2004; Martínez et al., 2005). However, operations such as removing petioles and sepals in strawberries, or rachis elimination in grapes, do not produce neither an increment in the respiration rate nor a higher ethylene production. This is probably because in these cases, the induced damage is minimal (Watada et al., 1996), moreover these products are non-climacteric and have a very low respiratory activity (Kader, 2002).

Early studies showed the effects of knives used for cutting on the stability of fresh cut products (Bolin et al., 1997). To reduce mechanical damage and the induced enzymatic browning and microbial development it is essential to cut the product with very sharp instruments. This will diminish the damage maintaining in better conditions the cellular integrity and avoiding the loss of internal fluids. For MFP lettuce it has been found that pieces cut with a blunt knife showed the highest respiration rate (Ahvenainen, 2000).The use of stainless steel reduces the risk of enzymatic browning avoiding the contribution of metallic ions (Cu^{+2}) that enhance it. It is also important the solidity of the industrial equipment, since, of being unstable, vibrations may alter the efficiency of the cut, causing cell and tissue damage and the consequent quality losses (Garg et al., 1990).

When it is needed to peel the product, like happens for citrus fruits, enzymatic peeling should be applied in order to minimise wounding. However, at industrial scale and for products like potatoes and carrots, mechanical, chemical and high pressure peeling still prevail. Abrasive peeling performed with water vapour or high pressure causes damage that will allow the attack of microorganisms (Wiley, 1994).

Likewise, the reduction in the size of the products has in many cases great incidence on its ulterior shelf life. For instance, in MFP lettuce it has been found that the smaller the size the bigger the respiration rate and ethylene production, which stimulates the biosynthesis of enzymes, as the phenyl alanine ammonia lyase (PAL), related with senescence (Hyodo et al., 1978; Martínez et al., 2005). Also, the fact of presenting a great exposed surface area induces a higher dehydration, and consequently a high weight loss. At the same time, damaged tissues usually synthesise different metabolites (as lignin and cumarins) in order to heal the induced wounds and as a way of defence from the pathogens presence. Consequently, it helps to avoid undesirable changes of the sensory quality of the product (flavour, aroma, colour, texture) and of their nutritive value (Kader, 1986; Varoquaux and Wiley, 1994).

At the same time, little is yet known on the effects of fresh-cut preparation, handling and treatments such as MAP on the nutritional quality of fresh cut vegetables and fruits. In reviews on the subject, it has been hypothesised that nutritional levels, especially of vitamin C, would be lower in fresh cut than in intact products (Klein, 1987; McCarthy and Matthews, 1994). Vitamins A and C and phenolic compounds could be inactivated as a result of wounding via exposure of interior tissues to light and air, enzymatic or chemical degradation in disrupted tissue, reaction with the products of lipid oxidation, wound ethylene or exposure to chlorine used for sanitation (Wright and Kader, 1997). Loss of vitamin C is also accelerated by water loss (Nunes et al., 1998) and its relative stability or degradation is highly dependent on temperature and commodity type (Klein, 1987).

Some treatments have been shown to help for keeping vitamin A and C levels in a few fresh cut vegetables and fruits. MAP helped to maintain higher levels of vitamins A and C in fresh cut broccoli (Paradis et al., 1996) but had little effect on vitamin A in peach and persimmon slices or vitamin C in persimmon and strawberry slices (Wright and Kader, 1997). Very high CO_2 concentrations (>20 kPa) may cause greater vitamin C degradation (Agar et al., 1997) while certain CO_2 levels may induce vitamin A to increase (Weichmann, 1986). The O_2 availability in the package headspace had a direct effect on the decrease in vitamin C content throughout storage. Consequently, ascorbic acid contents in fresh cut apples and pears have been shown to be affected by atmospheres rich in CO_2. A low degradation of ascorbic acid has recently been reported throughout 12 days of storage in 1-MCP-treated fresh cut pineapple. On the other hand, no changes in the phenolic content of fruits have been found due to storage atmospheres (Martin-Belloso and Soliva-Fortuny, 2006).

Levels of vitamins in fresh cut products after preparation and during handling and distribution in comparison to intact tissues are known for only few products. Some phenolic compounds in vegetables and fruits have been identified, but most of the data are very limited either to a single fruit or vegetable or to certain specific foods. Also, there have been limited reports on antioxidant activity of fresh vegetables and fruits. However, with recent increasing demand for fresh cut products, there is a trend to generate information related to biologically active compounds. It has been demonstrated that the increase in antioxidant capacity after wounding depends on the type of fruit or vegetable tissue (zucchini, white and red cabbage, iceberg lettuce, celery, carrot, parsnips, red radish, sweet potato and potatoes, etc.) Phenolic changes ranged from a 26% decrease to an increase up to 191%, while antioxidant capacity changes ranged from a 51% decrease to an increase up to 442% (Reyes et al., 2007).

PHYSIOLOGICAL DISORDERS AND THEIR CONTROL

In the intact whole vegetable products, the respiration rate can be used to estimate its shelf life in an approximate way. However, in the MFP products their physiology is altered so drastically that it is very difficult to make such predictions. The main physiological disorders more frequently found are described below.

Enzymatic Browning

One of the main causes of quality losses in MFP products and the major challenge in fresh cut fruits is enzymatic browning (Weller et al., 1997; Eissa et al., 2006; Toivonen and Delaquis, 2006). The presence of browning is basically due to the oxidation of phenolic compounds catalysed by the polyphenol oxidase enzyme (PPO), originating colourless quinones that later on polymerised forming melanins. These substances show brown, reddish or black coloration (Castañer et al., 1996). Many fruits (i.e., apple, peach, pear) and some vegetables (i.e., potato, artichoke) have high levels of preformed phenolic compounds. Following cutting, very rapid surface browning take place. This disorder has great importance for its high visual impact that decreases the sensorial acceptance by consumers and the marketability, as well as reduces the nutritional value of the products (Artés et al., 1999a). In tissue with initial low levels of preformed phenolic compounds (e.g., celery, lettuce) browning results from the induced synthesis and subsequent accumulation of phenolic compounds (Castañer et al., 1999). At the same time, wounding induces synthesis of enzymes involved in browning reactions or substrate biosynthesis. Thus, relative oxidase activities and substrate concentrations can affect browning intensity in diverse tissues and crops. In the biosynthesis of the phenolic compounds, the PAL plays an important role, whose activity is notably increased by the presence of ethylene. This kind of alteration appears very often in MFP products as apple, pear, peach, potato, mushroom or lettuce.

Sulphites have been used to control enzymatic browning. However, since sulphites are being forbidden, it is needed to find alternative products. Because the O_2 is needed for the browning reactions, the MAP technique with low O_2 and high CO_2 levels can contribute positively to avoid browning in MFP products, like it happens in lettuce or apple (Artés et al., 1999a; Nicoli et al., 1994). Also, the high CO_2 around the MFP product can inhibit the biosynthesis of phenolic compounds, substrates of the PPO, that habitually are induced in response to cutting damage (Ke and Saltveit, 1989). For that purpose, MAP with 2–8 kPa O_2 and between 5 and 15 kPa CO_2 (depending on the commodity and processing conditions) are usually recommended (Artés et al., 1999a). Partial vacuum packaging and/or the initial injection within the packages of certain gases mixtures (O_2, CO_2 and/or N_2), by using the active-MAP technique, frequently causes a quick modification of the atmosphere within package which allows prolonging the commercial life of MFP products. However, this technique should be applied with caution, since the excess of CO_2 and/or a too low concentration of O_2 usually cause damage. Other research works have shown the beneficial combined effect of high O_2 and CO_2 levels around MFP for keeping quality. For example, it has been recommended 80 kPa O_2 with 20 kPa CO_2 to inhibit the PPO activity and to control the enzymatic browning in MFP 'Iceberg' lettuce (Heimdal et al., 1994). High O_2 MAP controlled browning in peeled potatoes and avoided peel browning of longan fruit (Ahvenaninen et al., 1998; Tian et al., 2002). It has been suggested that high O_2 concentrations may cause substrate inhibition of PPO or, on the other hand, that high levels of formed quinones would generate feed-back product inhibition (Laurila et al., 1998; Day, 1996).

Other treatments have been used, with different success, to inhibit enzymatic browning as reducers (ascorbic acid), quelants (like EDTA) and acidulant agents like the citric and acetic acids (Vamos-Vigyazo, 1981; Dziezak, 1986; Sapers and Hicks, 1989; Sapers and Miller, 1992; Artés et al., 1999a; Castañer et al., 1996). Some antioxidants like 4-hexilresorcinol alone or combined with ascorbic acid and the use of MAP have been recommended for avoiding browning in sliced apples (Luo and Barbosa-Cánovas, 1997). Combined with potassium sorbate reduced changes in colour (L*, a* and b*) and microbial growth and did not affect sensory characteristics of fresh-cut mangoes. High humidity reached in-package atmosphere alleviated tissue dryness and was an important factor in the ability of the antibrowning solutions to prevent browning and decay (González-Aguilar et al., 2000). Even when they are effective to reduce the enzymatic browning, they should be applied with caution, since they may alter the sensory quality and the nutritional value of the products. In potato, browning may be controlled by the use of ascorbic or citric acid, but it seems that 'oxygen shock' treatments are also particularly effective (Limbo and Piergiovanni, 2005).

Another alternative in order to avoid enzymatic browning in fruits is the immersion of the slices in sugary solutions. For example, pineapple juice has been used to inhibit the enzymatic browning in MFP apples (Lozano-de-González et al., 1993).

Discolouration

Colour of fresh cut produce is probably the main quality attribute considered by consumers. Research in the past few years has focused on the prevention of discoloration (Salcini and Massantini, 2005). In green vegetables the senescence process usually leads to a yellow coloration of the tissues, due to the degradation of chlorophyll. Yellowing of green MFP products is not appealing to consumers and has a negative effect on sale of the product. It has been demonstrated that colour change from bright green to brown in fresh as well as in minimally fresh processed green vegetables is related to the formation of pheophytin. When chlorophyll loss its bound magnesium atom, which is substituted by hydrogen, forms pheophytin, an olive coloured pigment. More than 50% conversion of the chlorophyll to pheophytin can occur before a change of colour from bright green to olive brown could be observed (Lau et al., 2000). The maintenance of a low temperature and a high relative humidity, combined with atmospheres lowered in O_2 and moderately enriched in CO_2, are shown to be the main advisable techniques to delay this disorder.

In grated carrot, it is frequent the appearance of a superficial whitish layer that has been associated to the synthesis of lignin, natural healing of the tissues (Bolin and Huxsoll, 1991), although it has also been related to the dehydration of the dead cells (Avena-Bustillos et al., 1994). The resulting whiteness is an indicator of minimally processed carrots storability, as it results in an irreversible change of surface colour (Lavelli et al., 2005). To avoid this disorder, it has been intended the use of edible films of sucrose esters (Baldwin et al., 1995). The presence of a whitish appearance in the surface of tomato slices has been also attributed to dehydration (Artés et al., 1999b). This degradation due to surface dehydration has also been observed in carrot outer layers, leading in a reversible surface discoloration (Cisneros-Zevallos et al., 1997). It has been mentioned that calcium and combined treatment of calcium and ascorbic acid were effective in preventing discoloration of fresh cut apples (Drake and Spayd, 1983) and pears (Rosen and Kader, 1989). The first visually observed change at the cut surface of plant tissue is desiccation of the first layer of broken cells and one to a few additional subtending layers of cells. Suberisation of the next layers of cells occurs in many tissues, including potato and yam tubers, sweet potato and carrot roots, bean pods and tomato and cucumber pericarp (Kolattukudy, 1984; Walter et al., 1990). Avoiding desiccation at the cut surface of some fresh cut products is critical for maintaining acceptable visual appearance. For example, the development of 'white blush' on the surface of abraded 'baby' carrots, caused by desiccation of cellular remnants on the carrot surface, is the limiting factor in marketing the product despite the use of polymeric film packages (Cantwell and Suslow, 2002).

Another kind of discoloration is often shown in MFP lettuce. Browning on the leaf edge on non-photosynthetic tissues ('brown stain') has been associated to CO_2 concentrations higher than 2 kPa during cold storage. Also the presence of pink to brown stains ('russet spotting'), in the mybrid of the leaf is quite frequent, being related to an ethylene level higher to 0.1 ppm during cold storage. Low storage temperature as 1°C was very efficient to reduce leaf surface browning ('russet spotting' and 'brown stain'), but not leaf edge browning in 'Romaine' lettuce (Artés et al., 1999). These authors also reported in MFP lettuce that 200 ppm of citric acid was effective to reduce leaf edge browning, but not leaf surface browning. In fresh cut lemons, L* colour parameter tended to increase, with greater differences respect to the initial values when smaller the cut size. This increase could be due to tissue dehydration (Artés-Hernández et al., 2007).

Softening

Pectic enzymes that are released during cutting operations can cause tissue softening. Nevertheless, these processes are strongly influenced by other intrinsic factors such as cell wall morphology and composition, and their impact is mostly restricted to the parts of the fruit in contact with cut surface. Texture changes in most fresh cut fruits have been attributed to the rate of transfer of water, which leaks from the vacuoles of the damaged cells and diffuses through the tissue, as well as to the loss of water vapour form the fruit surface to the surrounding packaging atmosphere. It is therefore important to keep a constant low temperature and to provide proper packaging barriers for moisture retention. The damaging effects of processing are minimised at chilling temperatures because both degradative reactions and diffusion processes are reduced (Martin-Belloso and Soliva-Fortuny, 2006).

The O_2 and CO_2 levels do not usually have much effect on the texture of fresh cut fruits. However, the maintenance of a proper gas balance is important for avoiding damage to the tissues due to the toxicity produced by excessive CO_2 or anaerobic conditions. High CO_2 induced tissue breakdown and formation of large amounts of exudates, evidenced as droplets on the cellular surface and flooded intercellular spaces of fresh cut apples and pears (Martin-Belloso and Soliva-Fortuny, 2006). Bai et al. (2001) remarked the positive effect of moderate CO_2 (<15 kPa) on the development of translucency in fresh cut melon, which can be related to texture loss.

Ethylene produced by physical action of cutting is sufficient to accelerate softening, as it has been observed in banana and kiwifruit (Abe and Watada, 1991). In tomato, loss of firmness of slices during chilling storage was described by a first order reaction model that incorporated the stage of ripening of the fruit at harvest and both, temperature and time of storage (Lana et al., 2005). Softening of kiwifruit slices was delayed by 1 to 2 days at 5°C in response to 1-MCP treatment of either whole fruits before cutting or the slices after cutting. Concurrently, ethylene production rate was decreased by the 1-MCP treatment (Vilas-Boas and Kader, 2001).

The use of MAP is not always effective enough to keep firmness at harvest. A varied sensitivity towards softening has been observed depending on the product, like it has been shown in melon. Active MAP with high CO_2 and/or low O_2 and temperatures close to 0°C can retard the microbial growth and softening in MFP 'Amarillo' melon and, combined with calcium salts (chloride, lactate or propionate), it would help to kept a firmer tissue (7%) compared to that stored in air (Aguayo et al., 2001). Calcium appeared to both delay senescence related to membrane lipid changes and increase membrane restructuring processes in fresh cut carrots (Pichioni et al., 1996). Calcium also has been reported to maintain firmness of sliced strawberries, pears, shredded carrots and zucchini squash (Morris et al., 1985; Rosen and Kader, 1989; Izumi and Watada, 1995). Although calcium chloride has been most commonly used for this purpose, it has been shown that calcium lactate is as effective without imparting a bitter flavour as calcium chloride can at higher concentrations (Luna-Guzman et al., 1999).

Pithiness

This scarcely studied disorder is particularly important in celery and radish. Air spaces form in the cortex and are collectively called aerenchyma. The cortex becomes white, spongy or vacuolated, and appears dry. Aerenchyma develops through the lysis of parenchymatous cells of the cortex. This process is stimulated by several factors that trigger senescence early. Among them low-temperature stress, water deficit, flowering induction and radicular infections are the most important. This 'pith breakdown' is also accelerated by inappropriate preservation conditions after harvesting. It is thought that pithiness enhances O_2 transport within the plant since it is associated with the tolerance of some crops to anaerobic soils (Aloni and Pressman, 1999). When aerenchyma forms in celery petioles, it is characterised by the appearance of whitish regions and air spaces within the tissue, and is a major source of quality loss and decreased shelf life (Robbs et al., 1996; Saltveit and Mangrich, 1996; Cantwell and Suslow, 2002).

Ethylene signals the formation of aerenchyma in response to stress (either hypoxia or N deficiency) and induces a rise in activity of cell-degrading enzymes such as cellulase. Because of the complete disappearance of cell walls and protoplasts of the cortical cells that are affected in aerenchyma formation, leaving prominent gas-filled voids, it seems reasonable to suppose that a wide array of cell-degrading enzymes is involved. Indeed, cell-wall disappearance takes place relatively late in the process of cell lysis so that cellulase is unlikely to have a primary role. The rise in ACC synthase activity precedes that of cellulase activity in root tips, which is consistent with induction of the latter enzyme by ethylene. Inhibitors of ethylene action (Ag^+) or biosynthesis (AVG) effectively blocked aerenchyma formation in hypoxic roots (He et al., 1994).

Abscisic acid (ABA) may have a role in aerenchyma development since an increase in endogenous levels of ABA followed water stress and the development of pithiness. Exogenously applied ABA induced pithiness in detached celery petioles, with the degree of pithiness increasing with the increased ABA concentration. Flooding and nutrient deficiency lead to pithiness, but not as rapidly as water deficit (Saltveit and Mangrich, 1996). There are differences among varieties when compared their susceptibility to pithiness. It seems to be genetically controlled, being 'petiole without pithiness' recessively expressed (Whitlock, 1979).

Harvesting of celery appears to exacerbate aerenchyma formation during chilling storage. Minimal processing also seems to increase pithiness development and limits the use and shelf life of cut petiole segments. It has been also suggested that these stresses may induce pithiness through their effect on ABA and ethylene. Applying ethephon (an ethylene release agent) in the field stimulated parenchyma breakdown and induced pithiness, with the effect more pronounced in the basal region (Pressmann et al., 1983). Viña and Chaves (2003) mentioned that storage of the celery sticks at 0°C up to 27 days was effective in preventing the development of pithiness rather than at 10°C where between days 14 and 21 an increased pithiness was observed. In the same way, and compared to air, MAP of 6 kPa O_2 + 7 kPa CO_2 improved the sensory quality of celery sticks, avoided the loss of green colour, decreased the development of pithiness and retarded the growth of microorganisms after 15 days at 4°C (Gómez and Artés, 2005). Similarly, the commercial success of fresh-cut peach and nectarines slices has been limited due to their short life because of pit cavity. In this case, breakdown was diminished by MAP and the use of dipping in ascorbic acid and calcium lactate (Gorny et al., 1999).

Off-Flavours and Off-Odours

Development of off-flavours and off-odours are some of the most frequent reasons for depleted quality in fresh-cut fruits. MFP commodities are commonly packed in polymeric bags with selective permeability to CO_2, O_2 and water vapour, where a passive or active MAP is generated. When the steady state atmosphere reached is far from the appropriate one, it can take place the development of off-flavours and/or aromas due to a fermentative metabolism, caused by excessively low O_2 and/or high CO_2 levels (Artés et al., 2006). It should not be confused the presence of off-odours and the persistent off-flavours, with the strong aromas detected just in the moment of opening the packages, originated by the expansion of the aromas from the vegetable tissues in cold storage that quickly will disappear later opening, without affecting their sensory quality.

For instance, in grated carrot an increase in the chlorogenic acid content induced off-odours and off-flavours (Babic et al., 1993). It can be usually found a decrease in some volatile compounds and an increase in others during shelf life as it has been reported for fresh-cut 'Gala' apples after 14 days at 1°C, but it is not always associated with off-flavours development. Results suggested that perceived flavour intensity increased during the first few days post-cutting and packaging and then dissipated (Bett et al., 2001). In the same way some results suggest that stress adaptation of some fruit tissue (i.e., pineapples) resulting from fresh-cut involves a decrease in esters and others aroma compounds and synthesis of sesquiterpenes with phytoalexin properties (Lamikanra and Richard, 2004).

Treatments applied before cutting may induce off-flavours after shelf life as a fresh-cut product. For example it has been reported that 20 h of ethanol vapours applications in freshly harvested mangoes after a hot water treatment to simulate quarantine heat treatments induced off-flavours after 15 days at 7°C (Plotto et al., 2006). In the same way a reduction in aroma perception was found after 15 days when a humidified flow of ozone-enriched air was cyclically applied ($4 \pm 0.5\ \mu L/L$ O_3 for 30 min every 3 h) on fresh-cut 'Thomas' tomatoes stored at 5°C (Aguayo et al., 2006). On the other hand, it has been found that UV-C irradiation applied after cutting seems to reduce rancidity in fresh-cut cantaloupe melon after 7 days at 10°C suggesting that cutting fruits under UV-C improved product quality (Lamikanra et al., 2005).

Translucency

Translucency is a physiological disorder also named 'glassiness' or 'watercore' characterised by the alteration of flesh texture to become dark and glassy and the presence of an early over-maturity. Flesh translucency is an important problem in some fruit for fresh market and affected fruits are more susceptible to postharvest mechanical damage. The cellular free spaces are filled with liquid, giving to the tissues a transparent or vitreous appearance, as it has been observed for melon, water melon, cucumber, tomato, papaya (O'Connor-Shaw et al., 1994) and pears (Soliva-Fortuny et al., 2002). Some preharvest conditions predispose fruits to translucency being highly important weather conditions (Paull and Reyes, 1996).

When this disorder takes place, the MFP products usually develop unpleasant aromas and the flavour is poor. In melon it has been found that translucency decreases under 15 kPa CO_2 enriched atmospheres (Portella and Cantwell, 1998). This fact is generally associated to a higher activity of the catalase and to α and β-galactosidase enzymes, which cause modifications in the membrane galactolipids increasing its permeability. Development of translucency frequently occurs in MFP tomato and melon. Its origin has not been yet elucidated although it has been related to calcium deficiency (Odet and Dumoulin 1993; Chatenet et al., 2000) and treatments that accelerate ripening and senescence like high storage temperatures and certain types of cut (cylinders vs slices; Aguayo et al., 2004). Additionally it is also related to the type of melon and pulp colour, and for example, 'Amarillo' cv is less sensitive to translucency than 'Cantaloupe' or 'Galia' cv. (Aguayo et al., 2003). Bai et al. (2001) highlighted the positive effect of moderate CO_2 (<15 kPa) on the development of translucency in fresh-cut melon, which can be related to texture loss.

Chilling Injuries

Since fresh-cuts have been subjected to severe physical stress and are more perishable than intact products, they should probably be stored at temperatures lower than that recommended for the intact commodities (Karakas and Yildiz, 2007). However, many plant foods commodities are sensitive to chilling injuries, a physiological disorder induced by low temperatures but above the freezing point. However it is unusual to find this kind of physiological disorders in MFP chilling sensitive products. This fact is commonly related to the short duration of the shelf life, which is usually from 1 to 2 weeks. In this period it is quite difficult to overpass the threshold or the latency period to manifest common symptoms of chilling injuries (Artés-Hernández et al., 2007). Moreover, it has been demonstrated that MAP increases the chilling tolerance of some products like cucumber (Karakas and Yildiz, 2007).

The case of the tomato slices translucency could be an exception, since a discoloration considered as a chilling injury was found during storage at 0°C, although it is not completely confirmed yet. Lana et al. (2006) described that slices obtained from fruit at red stage got translucent faster than from fruits at breaker stage and the intensity of translucency was also higher for more ripe fruit. The storage temperature (between 5 and 13°C) did not influence the development of translucency. At the same time, some results suggest that low ethylene levels enhance chilling injury and that MAP can provide good quality tomato slices with a shelf life of at least 2 weeks at 5°C (Hong and Gross, 2001). The rate of ethylene production in ethylene pretreated tomato slices (immediately and 3 days after slicing) tended to show a negative correlation with chilling injury (Ji-Heun and Gross, 2000).

MICROBIAL GROWTH AND THEIR CONTROL

Microbial decay is the major source of spoilage of fresh-cut product (Babic et al., 1996) since washing and chlorinated water dips only partially remove the microorganisms intrinsic to produce (Garg et al., 1990). The presence of damaged cells and the loss of cellular components during processing operations provide optimum conditions for the development of microorganisms. The type and species, as well as the microbial levels in the MFP products varies with the fruit or vegetable, the cultivation practices and the hygienic conditions during handling and processing, being the temperature during shelf life the essential factor. Chlorinated water with 50 to 150 ppm of NaClO, as it is frequently used in the MFP factories for disinfection, could be effective to avoid microbial development, but it is impossible to eliminate all the microorganisms present in the commodities. Besides, because chlorine reacts with the organic matter decreasing the concentration of the active form (hypochlorous acid) it is not always possible to maintain a homogeneous concentration in the washing step. For that reason, an effective control of chlorine concentration and pH levels should be executed (Artés-Hernández and Artés, 2005).

It is crucial to carry out microbial preventive controls in MFP industries. Acting directly on the different stages of product processing will guarantee the safety for consumers. Consequently, it is indispensable to implement accurate hazard analysis and critical control points (HACCP) systems in all the MFP factories. Currently new approaches must be implemented including audit of HACCP systems, risk communication and consumer information. The development of reliable traceability systems to establish the origin/mode of production of food products should provide information to each stakeholder, easily incorporated in a bar code (Gorny, 2001; Suslow, 2003; Artés, 2004).

Bacteria as well as fungal and yeasts proliferate in the periphery and interior of the damaged tissues and dead cells, enhanced by the presence of cellular fluids. Due to this it is essential to rinse, centrifuge or drying with cold air the processed product with the purpose of decreasing the surface humidity. However, dehydration, shrivelling and abrasion must be avoided (Bolin and Huxsoll, 1991). It should be emphasised that doing a complete microbial analyses of MFP products often requires several days, what supposes that the results are obtained once the product has been marketed. For that reason, prevention and self-controls should be exhaustive in the industrial factories and for each product. In this way, as a really high-priority task, it is convenient to carry out the development of microbiology predictive models for MFP products.

There is a real concern over the possible regulatory restriction of chlorine use in processing waters. Research has showed the formation and persistence of toxic compounds called trihalomethanes that are carcinogenic. A potential replacement under revision is O_3 because of its strong oxidising power. The O_3 is a gas authorised in several countries for direct contact with harvested produce. The recent recommendation to the FDA by an expert panel supporting their classification as a generally recognised safe disinfectant for foods offers a way for the industry to favour safety of produces when applied along with good manufacturing practices (Kim et al., 1999). It has been reported that O_3 enriched atmospheres retarded the growth of pathogens like *Sclerotinia sclerotiorum*, *Botrytis cinerea* or *Rhizopus stolonifer* (Liew and Prange, 1994; Sarig et al., 1996). An O_3-enriched air flow cyclically applied favoured good appearance and overall quality in a ripening-inhibited tomato slices after 15 days at 5°C. The O_3 showed a strong effectiveness in reducing microbial populations, being more effective on bacterial rather than on fungal reduction (Aguayo et al., 2006).

Also, to the well-known fungistatic effect of CO_2 used in the conventional MAP technique, it is remarkable the use of natural compounds, such as hexanal, 2-(E)-hexenal, hexyl acetate and citrus essential oils, to improve the shelf-life and the safety of minimally processed fruits, even when their mechanisms of action is not yet completely known (Lanciotti et al., 2004). Gamma radiations, edible films coatings and biological controllers, like the yeast *Candida quiulliermondii* to control *Penicillium* sp. have also been studied (Watada et al., 1996). It has been found that the use of high-CO_2 atmospheres in tolerant products as strawberries (30 kPa) allow the control of fungal development (Wills et al., 1998).

In the same way, the effect of high O_2 atmospheres (50–90 kPa) has also been evaluated, alone or combined with moderate to high CO_2 (10–20 kPa). It has been found that it was possible to reduce growth of yeasts and bacterias that usually alter the quality of MFP vegetables (Amanatidou et al., 1999). For example, super atmospheric O_2 (80–100 kPa) into MAP packages alleviated tissue injury, reduced microbial growth and was beneficial in maintaining quality of fresh-cut baby spinach up to 12 days at 5°C (Allende et al., 2004) or in MFP mixed salads (Allende et al., 2002). It also has been mentioned how 50 or 80 kPa O_2 combined with 15 kPa CO_2 maintained the main sensory quality attributes and inhibited growth of the spoilage microorganisms and *Enterobacteriaceae* in MFP bell peppers stored up to 10 days at 5°C (Conesa et al., 2007).

The germicidal properties of the UV radiation in the range of 200–280 nm have been used in the fruit juices industry as well as for disinfection of water supplies and food contact surfaces because it penetrates only 50–300 nm into the tissue. Recently, the use of UV-C at 254 nm alone or combined with MAP was proposed to reduce microbial growth and keeping quality in some MFP fruit and vegetables which seems to be a very promising technique (Erkan et al., 2001; Marquenie et al., 2002; Allende and Artés, 2003a, b; López-Rubira et al., 2005; Vicente et al., 2005; Artés et al., 2005).

It must be pointed out that there is not yet a common legislation in the European Union that regulates the maximum limits of bacterial counts tolerated in MFP products, what supposes a legislative deficit not very acceptable when the security of the consumers is concerned. The only exception is for *E. coli* and *Salmonella* for whose tolerance limits have been established (Commission Regulation 2073/2005, 2005). In France it has been established in 10^7 cfu/g, being from 4.7 log cfu/g at production level to 7.7 log cfu/g at consumers level (CNERNA-CNR, 1996), in agreement with the approaches adopted by Belgium producers (Debevere, 1996). The maximum microbial limit fixed by the Spanish legislation (RD 3484/2000, 2001) for aerobic bacterial growth is 10^5 cfu/g in the processing day, and 10^7 cfu/g in the consumption day. Additionally *Listeria monocytogenes* and *Salmonella* spp. must be absent in a sample of 25 g.

Tests for specific pathogens are needed to evaluate the microbial risk of a particular product that seems to be the main reason for what no microbiological standards for MFP products exists in the USA. It must be clearly stated that food safety is based on prevention strategies through good handling and manufacturing programs and HACCP approach (Cantwell and Suslow, 2002).

Although at temperatures below 5°C mesophilic bacteria can hardly grow, the psicrotrophic ones like *Yersinia enterocolitica*, *Salmonella* spp., *Listeria monocytogenes* and *Aeromonas hydrophila*, can develop, constituting a real, although fortunately not very common, risk for consumers safety in the MFP products commercialised at low temperatures (Brackett, 1994). Other bacterial types as *Pseudomonas* sp. and *Erwinia* sp. considered not very dangerous for consumers, are more habitual in MFP products (Varoquaux and Wiley, 1994), causing the 'bacterial rot soft' frequently isolated in MFP products like lettuce salads (Artés et al., 1999).

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