review

Role of microbiological and physiological spoilage mechanisms during storage of minimally processed vegetables

P. Ragaert, F. Devlieghere*, J. Debevere

Ghent University, Faculty of Bioscience Engineering, Department of Food Safety and Food Quality, Laboratory of Food Microbiology and Food Preservation, Coupure Links 653, 9000 Ghent, Belgium

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Abstract

Minimally processed vegetables (MPV) are economically important commodities due to a combination of factors such as convenience, healthiness and desirable sensory characteristics. These commodities are susceptible to microbiological invasion due to the presence of cut surfaces causing both microbiological and physiological mechanisms to be possible limitations for the sensory shelf life. This review evaluates the role of microbiological activity in the development and changes of different sensory quality factors (visual, flavour, and textural quality) of minimally processed vegetables and evaluates the possible interaction with physiological mechanisms, taking into account important preservation techniques such as storage temperature and atmospheric conditions.

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1. Introduction

In recent decades, consumers have been living in a society where convenience and high quality food products are at a premium. One of the responses of the food industry to this trend has been the production of minimally processed vegetables. These vegetables combine their fresh-like and healthy characteristics (preserved during storage by a natural packaging system) with a minimal time of preparation before consumption both at the consumer and catering levels (Ahvenainen, 1996; Watada et al., 1996; Ragaert et al., 2004b). This has led to a rapid increase in the type of minimally processed vegetables offered and consequently to high sales. Data from a supermarket group in Belgium has indicated that the quantities (kg) of minimally processed vegetables (soup vegetables not included) sold in the period 1999–2004 increased by 57%. This corresponded to an increase...
in sales of 109% (Anonymous, 2005). Vegetables for making soup also belong to the group of minimally processed vegetables, but are not considered in this review as they require further processing by the consumers before consumption. This review focuses on the following minimally processed vegetables, since they have been the topic of research on spoilage: shredded leafy vegetables such as lettuce and endive, shredded carrots, shredded artichoke, cut broccoli, cut cauliflower, shredded cabbage, shredded chicory endive and shredded bell peppers.

Minimally processed vegetables have a physical structure which is susceptible to microbiological invasion. It means that both microbiological and physiological activities could play a role in quality degradation during storage. This review describes the degradation of sensory quality during storage of minimally processed vegetables focusing on visual defects, degradation of texture and presence of off-odours and off-flavours. It focuses on evaluation of the role of microbiological activity in the development and changes in these different sensory quality factors and of the possible interaction with physiological mechanisms.

2. Microbiological activity on minimally processed vegetables

2.1. Activity related to tissue structure

Vegetables consist mainly of water, resulting in a high water activity (>0.99). The intracellular pH, being another important intrinsic factor, ranges for most minimally processed vegetables from 4.9 to 6.5 (Lund, 1992). These properties allow the growth of micro-organisms from the moment that nutrients become available. Under natural conditions, the outer layer of the plant tissue consists of a hydrophobic surface providing a natural barrier for micro-organisms (Lund, 1992). The surface of plants is however subjected to external stress factors before, during and after harvest due to weather, insects, birds, rodents, farm implements, harvesting and processing which can all lead to physical damage (Snowdon, 1990). Moreover, microbiological damage of intact surfaces is possible if these micro-organisms can change the physicochemical properties of the surfaces by producing bio-surfactants (Laycock et al., 1991).

Due to damage of the surface, nutrients are released from the plant tissue, which can be used by micro-organisms. It has been shown in the case of Pseudomonas fluorescens that densities of this micro-organism are directly correlated with the amount of sugars present in leaves of vegetable plants (e.g. bean leaves) and that these sugars are the limiting factor with regards to colonisation (Mercier and Lindow, 2000). In relation to the presence of damaged areas, Babic et al. (1996) found that microbiological populations were situated on cut surfaces of spinach. This is similar to the findings of Brocklehurst and Lund (1981) who reported that after inoculation of celery with fluorescent pseudomonads, soft rot could not develop on unwounded tissue, possibly due to limits to proliferation.

Wounded areas on plant tissue provide a better substrate for microbiological growth by providing nutrients (King et al., 1991; Zagory, 1999), and the properties of the tissue determine which micro-organisms will be active. Some of these micro-organisms produce pectinolytic enzymes (see Section 4.3) which degrade the cell structure and as such provide more nutrients for microbiological activity. Generally, commodities that are susceptible to a high degree of nutrient release tend to have high microbiological counts. Moreover, physiological ageing of commodities could increase microbiological counts (Jacques and Morris, 1995; Zagory, 1999; Cliffe-Byrnes and O’Beirne, 2005).

Because of the use of blades during processing, minimally processed vegetables contain many wounded areas resulting in localised microbiological proliferation. The amount of cells damaged during minimal processing can be reduced by using appropriate peeling and cutting methods (Kader and Mitcham, 1997; Barry-Ryan and O’Beirne, 1998, 2000; Watada and Qi, 1999). Besides microbiological activity, the presence of damaged areas results in a stress response of the produce itself, such as an increase in the respiration rate and ethylene production, resulting in faster metabolic rates (Howard et al., 1994; Watada et al., 1996; Saltveit, 1999; Laurila and Ahvenainen, 2002; Surjadinata and Cisneros-Zevallos, 2003). Changes in the respiration rate and corresponding metabolism of the plant contribute to resistance of plants to mechanical stress, including limiting microbiological invasion of the wound site (Kang and Saltveit, 2002; Lamikanra, 2005).

Besides these changes in metabolic rates, damage leads to exposure to air, desiccation and exposure of enzymes to their substrates, all leading to quality degradation (Klein, 1987; King and Bolin, 1989; Roura et al., 2000).

2.2. Micro-flora of minimally processed vegetables

In general, total counts of microbiological populations on minimally processed vegetables after processing range from 3.0 to 6.0 log cfu/g. The dominating bacterial population during low temperature storage mainly consists of species belonging to the Pseudomonadaceae (especially P. fluorescens) and Enterobacteriaceae (especially Erwinia herbicola and Rahnella aquatilis), besides some species belonging to the lactic acid bacteria (especially Leuconostoc mesenteroides) (Lund, 1992; Nguyen-the and Carlin, 1994; Vankerschaver et al., 1996; Bennik et al., 1998). In contrast with bacteria, many different yeast species of comparable quantitative importance have been identified in minimally processed vegetables, including species of Candida, Cryptococcus, Rhodotorula, Trichosporon, Pichia and Torulaspora (Nguyen-the and Carlin, 1994). Moulds are less important in minimally processed vegetables due to the intrinsic properties such as a slightly acid to neutral pH favouring bacteria and yeasts which will overgrow moulds (Magnunson et al., 1990; King et al., 1991; Lund, 1992; Moss, 1999; Giménez et al., 2003; Tournas, 2005).

The incidence and growth of pathogens on minimally processed vegetables is not included in the scope of this review, see reviews by Brackett (1999), Francis et al. (1999), Seymour and Appleton (2001), Everis (2004), and Sivapalasingam et al. (2004). Minimally processed vegetables can also be contaminated with human pathogens, present from before, during or after harvest. Possible contamination sources are seed, soil, irrigation water, animals, manure/sewage sludge use, harvest-
ing, processing and packaging. Bacterial, viral and protozoan pathogens have been implicated in produce-related outbreaks. Good agricultural practices (GAP), good manufacturing practices (GMP) and a HACCP type approach should be followed to minimise the risk of contamination and outgrowth (Brackett, 1999; Francis et al., 1999; Seymour and Appleton, 2001; Evers, 2004; Sivapalasingam et al., 2004).

In this regard, the cold chain should be maintained both in production and in storage to minimise microbial development and ensure optimal shelf life extension (Legnani and Leoni, 2004). It is also important to notice that techniques aimed at decreasing the activity of spoilage micro-organisms, might possibly enhance the growth of pathogens due to a reduction in competing flora. Moreover, safety problems can occur when a disturbing level of pathogenic flora is reached before detection of sensory defects (Brackett, 1999; Francis et al., 1999; Giménez et al., 2003; Evers, 2004).

3. Preservation to influence quality degradation

The information gathered in this review is mostly based on studies investigating one or more preservation techniques for minimally processed vegetables, since these studies monitor microbiological and/or physiological changes during storage time. For this reason, an overview of these preservation techniques is given prior to the discussion of the role of microbiological spoilage mechanisms on sensory quality during storage of minimally processed vegetables. Moreover, this section of the review focuses on the effect of these techniques on microbiological and physiological activity.

3.1. Temperature and atmosphere

In general, preservation techniques are often only effective if products with an optimal initial quality are used as raw material. The two most used preservation technologies for minimally processed vegetables are low temperature and controlled (CA) or modified (MA) atmospheres. Both result in a decrease in the respiration rate of vegetables resulting in an extension of shelf life (Day, 1993; Watada et al., 1996). With regards to microbiological activity, the effect of modified atmospheres on microbiological growth is not consistent and the storage temperature rather than gas composition tends to control microbiological growth (Zagory, 1999). Similarly, the benefits of modified atmospheres for maintaining quality are not consistently related to a reduction in growth of mesophilic flora (Nguyen-the and Carlin, 1994).

The inability of lower O2 and higher CO2 concentrations to consistently decrease microbiological growth has also been shown by Bennik et al. (1998) who found no differences in growth on a synthetic medium between 1.5% O2 and 21% O2 at 8 °C for both Pseudomonas spp. and Enterobacteriaceae. The effect of increasing CO2 concentration on the growth rate was not consistent. In the case of Enterobacteriaceae, significant decreases were found with 20% CO2 while in the case of Pseudomonas spp., significant differences in growth rate could be observed from 5% CO2. O2 concentrations below 1.5% and CO2 concentrations higher than 20% however, exceed the limits of tolerance for most minimally processed vegetables, resulting in physiological damage with subsequent effects on sensory quality (Zagory, 1999; Giménez et al., 2003). The limits of tolerance to low O2 levels and high CO2 levels, however, are subject to several variables, such as type of produce, cultivars, temperature, physiological condition, maturity and previous treatment (Day, 1993; Toivonen, 1997).

The use of high O2 concentrations (80–90%), in combination with moderate CO2 concentrations (10–20%) has been investigated as an alternative to inhibit micro-organisms using modified atmospheres without a negative impact on physiology processes (CO2-injury), (Amanatidou et al., 1999; Jacxsens et al., 2001; Allende et al., 2004). Research with such atmospheres has revealed a variable effect on micro-organisms. Overall quality and sensory quality could however be improved due to the absence of enzymatic browning in those commodities sensitive to browning (Jacxsens et al., 2001; Allende et al., 2004). Moreover, high O2 concentrations seemed to alleviate injuries caused by high CO2 concentrations (15–25% CO2) (Jacxsens et al., 2001; Allende et al., 2004).

Besides temperature and gas conditions, maintenance of an optimal relative humidity is also very important in a good storage or packaging system. This can be obtained by choosing a packaging film for minimally processed vegetables with an appropriate water vapour permeability. Packages maintaining an optimal relative humidity (RHopt) prevent moisture loss (if RH < RHopt) or mould growth (if RH > RHopt) (Zagory and Kader, 1988). RHopt for most produce ranges between 90 and 100% (Schlimme and Rooney, 1994; Paull, 1999). Moisture losses of 3–6%, due to the RH being too low usually result in marked deterioration of quality (Day, 1993). Paull (1999) mentions a maximum allowable moisture loss of 3% in the case of lettuce.

Due to low temperature storage, the microbiological population on vegetables is expected to be dominated by psychrotrophic micro-organisms (Zagory, 1999). This was shown by Giménez et al. (2003) and Hong and Kim (2004), who also found that these micro-organisms are able to grow in the same incubation conditions suitable for mesophilic micro-organisms. However, some other studies which have involved measurement of both the mesophilic (incubation at 30–35 °C for 1–2 days) and psychrotrophic (incubation at 3.3 °C for 4–10 days) microbiological counts during low temperature storage, have shown that selection towards psychrotrophic micro-organisms in some cases could take place before packaging (e.g. during cold storage before processing) and therefore, that inconsistent differences between these two counts can be observed (Garg et al., 1990; Barriga et al., 1991; Babic et al., 1996; Li et al., 2001).

3.2. Other preservation techniques

Besides the use of low temperatures, alone or in combination with modified atmosphere packaging, other techniques can be used to decrease the rate of quality degradation in minimally processed vegetables during storage. These techniques aim to decrease the initial level of microbiological contamination and/or to slow down microbiological and physiological
activity during storage. There has been varying success with the application of one or a combination of several techniques during processing such as use of chemicals (e.g. chlorine-containing compounds), electrolysed oxidizing water, mild thermal treatments, irradiation, electric pulses, ultrasound, light pulses, UV-C treatment, anti-browning agents, acidifying agents, calcium solutions, natural antimicrobials, edible film coatings and active packaging (Nguyen-the and Carlin, 1994; Bennik et al., 1996; Kelly et al., 1998; Francis et al., 1999; Izumi, 1999; Kim et al., 1999; Han et al., 2000; Prakash et al., 2000; Li et al., 2001; Seymour and Appleton, 2001; Tomás-Barberán and Espín, 2001; Kang and Saltveit, 2002; Li et al., 2002; Seymour et al., 2002; Singh et al., 2002; Allende and Arts, 2003; Emmambux and Minnaar, 2003; Devlieghere et al., 2004; Everis, 2004; Ragaert et al., 2004a, Gomez-Lopez et al., 2005).

The success of these techniques depends on different factors. The efficacy of decontamination methods is reflected in the microbiological reduction obtained and even more important maintenance of this reduction during storage. This will depend on the sensitivity of the micro-organism and the accessibility of the agent or the treatment to the microbiological populations. Damaged areas play an important role in the accessibility of agents or treatments by protecting micro-organisms from particular preservation techniques (Adams et al., 1989; Watada et al., 1996; Takeuchi and Frank, 2001). Micro-organisms can grow rapidly after treatment due to the availability of nutrients in these areas. A second factor determining the success of preservation techniques is the degree to which undesirable effects occur, such as softening of plant tissue or discoloration due to application of the technique. Moreover, physiological damage due to treatments could increase microbiological activity because the fraction of surviving micro-organisms will proliferate more quickly due to an increased amount of available nutrients. A reduction in epiphytic microflora may result in an increase in the potential growth of pathogens (Bennik et al., 1996; Zagory, 1999; Li et al., 2002). A third factor relates to the effect of these techniques on nutritional quality and on the possible formation of by-products which could have implications for human health, as in the case of certain chlorination disinfection by-products such as trihalomethanes and halocetic acids (Chang et al., 2000; Nieuwenhuijsen et al., 2000). The success of preservation techniques will of course also be related to economic factors such as expenses related to the equipment and the extension of the processing chain due to the application of the preservation treatment. Research on decontamination techniques too often focuses on only one of the above-discussed factors, usually concentrating on microbiological reduction.

4. Degradation of sensory quality in relation to microbiological contamination

4.1. Visual defects

Visual characteristics have served as a primary means for quality differentiation at purchase. Visual defects can already be present directly after packaging due to causes originating before, during or after harvest, such as insect damage, plant diseases, adverse weather conditions, physiological disorders due to inadequate plant nutrition, or improper handling and mechanical damage incurred during harvesting and handling (Shewfelt, 1990).

One visual defect occurring during storage of some minimally processed vegetables is enzymatic browning in which two plant enzymes play a major role: PAL (phenylalanine ammonia lyase), a key enzyme in phenolic biosynthesis and PPO (polyphenoloxidase), which converts these phenols into quinones, which rapidly condense to produce relatively insoluble brown polymers (melanins) (Martinez and Whitaker, 1995; Tomás-Barberán and Espín, 2001). Bruised or ruptured cells in damaged areas of tissue result in cellular enzymes such as PAL and PPO coming into contact with substrates, with subsequent phenol oxidation and eventually melanin formation (King and Bolin, 1989). Moreover, wounds induce changes in phenolic compound composition such as increases in chlorogenic acid, dichaefoyle tartaric acid, and isochlorogenic acid (Ferreres et al., 1997; Gil et al., 1998; Kang and Saltveit, 2002; Lamikanra, 2005). Enzymatic browning can be delayed by modified atmospheres whether or not in combination with anti-browning agents (Heimdal et al., 1995; López-Gálvez et al., 1996; Gil et al., 1998). Although, these studies did not involve microbiological counts, it should be noted that pectinolytic micro-organisms could break down cell walls resulting in stress-related exposure of enzymes and substrates, which also could lead to enzymatic browning.

The effect of microbiological activity on some other visual defects has been investigated in several papers. Some of these reports aimed to establish a limit for the psychrotrophic count on minimally processed vegetables at which microbiological degradation becomes obvious. Nguyen-the and Prunier (1989) dipped cut chicory leaves in suspensions of bacteria (7–9 log cfu/ml) belonging to fluorescent Pseudomonads and known to cause ‘soft rot’ symptoms. Visible decay of the dipped leaves was observed after 4 days at 10°C under air with a corresponding bacterial count of 8.2 or 8.8 log cfu/g. Moreover, no decay could be observed on dipped leaves where bacterial counts reached 7.7 log cfu/g or less after storage for 7 days at 10°C under air. The same trend was found by Giménez et al. (2003) who found unacceptable changes of appearance during storage of minimally processed artichoke at 4°C under air at day 15, corresponding to a psychrotrophic count exceeding 8 log cfu/g at day 13 and reaching 8.8 log cfu/g at day 15. Li et al. (2001) also found that the visual quality of packaged cut iceberg lettuce stored at 5°C (packages closed under ambient atmosphere; no information about gas conditions) became unacceptable at day 14, corresponding to aerobic psychrotrophic and yeast counts of 8.8 and 6.4 log cfu/g, respectively. Visual quality of packaged cut lettuce (no information on gas conditions) became unacceptable after 10 days of storage at 2°C when the total microbiological counts and yeast counts reached 8 and 5 log cfu/g, respectively (King et al., 1991).

Other reports have shown that a psychrotrophic count of at least 8 log cfu/g is not sufficient for visual defects (Jacques and Morris, 1995). Moreover, the use of modified atmospheres could inhibit visual defects. This was shown by Bennik et al. (1998)
who found that cut chicory endive stored under CA (1.5% O₂, 20% CO₂, balance N₂) had a good appearance after 9–13 days of storage at 8 °C even though the total mesophilic count and Enterobacteriaceae reached 8–8.5 log cfu/g, while Pseudomonas spp. reached 7.5–8.0 log cfu/g. Samples stored under air were already unacceptable after 6 days due to soft rot and brown discoloration with 50% higher maximum specific growth rates for both Enterobacteriaceae and Pseudomonadaceae. The beneficial effects of modified atmospheres towards maintenance of visual quality were also shown by Barriga et al. (1991), King et al. (1991) and Hong and Kim (2004). However, they reported no relationship between microbiological counts and modified or controlled atmospheres, suggesting that these atmospheres had a retarding effect on physiological activity.

In many commodities, the rate of browning determines the sensory shelf life (Jacxsens et al., 2002; Jacxsens et al., 2003). Lower O₂ concentrations cause slower rates of enzymatic browning, with the result that sensory quality factors other than browning become more important with regards to shelf life. Such is the case with soft rot caused by high microbiological counts (>8 log cfu/g).

The degree of interaction between microbiological and physiological activity can also depend on the type of blades used to cut vegetables. Visual appearance of carrot slices, cut by blunt blades, packaged under MA at 8 °C for 10 days became unacceptable when total aerobic counts exceeded 8 log cfu/g (Barry-Ryan and O’Beirne, 1998). CO₂ concentrations were between 30 and 35%, while O₂ concentrations were between 2.5 and 5.0%. Samples having undergone less stressful conditions during processing (for instance, where razor blades were used with consequentially better integrity of the tissue) remained visually acceptable over 10 days, and total aerobic counts never exceeded 8 log cfu/g. At day 10, the maximum CO₂ concentration during storage of these samples was 30%. Although, this concentration is higher than the tolerance level of most minimally processed vegetables, no defects in appearance and aroma were detected. As has already been mentioned, the limits of tolerance to low O₂ and high CO₂ levels are dependent on different factors.

With regards to these limits of tolerance, O₂ concentrations that are too low or CO₂ concentrations too high could induce physiological disorders independent of microbiological activity. During storage of minimally processed artichoke at 4 °C, Giménez et al. (2003) found that packaging leading to high CO₂ concentrations (>25%) resulted in an unacceptable visual quality with a corresponding psychrotrophic count between 6 and 7 log cfu/g. The high CO₂ concentration resulted in physiological browning and necrosis (CO₂ injury). Such high CO₂ concentrations were also found from day 21 with shredded carrots stored at 4 °C resulting in a slimy appearance and accumulation of fluid inside some bags (Hao et al., 1999). The psychrotrophic count on day 21, dominated by yeasts, was 7.2 log cfu/g, which could also have been responsible for these defects. García-Gimeno and Zurera-Cosano (1997) investigated the sensory shelf life of a packaged ready-to-eat vegetable salad based on textural and visual quality. At 4 °C, these quality factors remained acceptable during the whole storage period (8.5 days), while at 15 °C, they became unacceptable after 84 h (3.5 days). When this spoilage became observable, the count of lactic acid bacteria exceeded 6 log cfu/g. It should be noted however that the O₂ concentration at 4 °C reached 0% at 60 h, which would normally result in bad taste or odours independently of microbiological activity, resulting in a shelf life determined by bad taste or odours before visual and textural quality becomes unacceptable.

4.2. Off-odours/off-flavours

A review of volatile and non-volatile compounds present in minimally processed vegetables is not given here. This has previously been done for fruits and vegetables by Toivonen (1997) and Cadwallader (2005), primarily from a physiological point of view. The target of this discussion is spoilage-related compounds that are produced during storage of minimally processed vegetables, focusing more on compounds of possibly microbiological origin.

Off-flavours can be detected by high performance liquid chromatography (HPLC), gas chromatography (GC), electronic nose systems or sensory analysis by a trained panel (Schaller et al., 1998; Wilkes et al., 2000; Brinkman, 2002). Detection of volatile compounds by GC can be by both direct analysis of sample homogenates (e.g. after solvent extraction) and headspace analysis, depending on the aim of the research (Wilkes et al., 2000). In the case of headspace analysis of either the sample or the sample homogenate, volatile compounds can be sampled by taking a small amount of gas out of the headspace (static), by flushing the headspace with a gas that is led through an adsorption tube (dynamic) or by solid phase microextraction (SPME) (Elmore et al., 1997; Wilkes et al., 2000; Holt, 2001). These different analytical techniques can lead to different results when analysing the same type of product. This has been shown in the case of fruit by Boschetti et al. (1999) and Forney et al. (2000).

Mechanical wounding of vegetables enhances a diverse array of enzymatic pathways, associated in many cases with generation of volatiles (Toivonen, 1997). However, research on changes in volatile and non-volatile compounds in minimally processed vegetables during storage in relation to microbiological activity is scarce. Some reports have involved the effect of low O₂ and/or high CO₂ concentrations on production of fermentative metabolites in combination with microbiological analysis. López-Gálvez et al. (1997) correlated increases in CO₂ concentrations with increases in fermentative metabolites (ethanol and acetaldehyde) during storage of packaged salad products at 5 °C. The presence of ethanol and acetaldehyde was in turn correlated with off-odour scores from a trained panel. Differences in off-odours between different salad products could not be related to the total aerobic count. O₂ concentrations that are too low can also result in fermentative metabolites, as described by Smyth et al. (1998) who found ethanol in the headspace of cut iceberg lettuce when 0.3–0.4 kPa O₂ and 0.6–0.8 kPa O₂ was reached during storage at 5 and 10 °C, respectively.

Fermentative metabolites could be present directly after packaging due to a wounding response, as suggested by Smyth et
al. (1999) in the case of cut carrots. Hagenmaier and Baker (1998) found increases in ethanol concentrations during storage of bagged salad at 5.5 °C. Increases in ethanol levels during storage could be caused by fermentative reactions due to high CO₂ (10–23%) and/or low O₂ concentrations (0.5–0.6%) or due to microbiological activity (mesophilic counts ranged from 7.2 to 7.9 log cfu/g). Microbiological production of ethanol was shown by Jacksens et al. (2003) and Ragaert et al. (2006a) on a simulation medium of mixed-lettuce agar. Moreover, microbiological production of other volatile organic compounds such as 2-methyl-1-butanol and 3-methyl-1-butanol has been demonstrated on this simulation medium (Ragaert et al., 2006a). This production was detected from a count of 6.7–7.1 log cfu/cm² in the case of bacteria and 5.0 log cfu/cm² in the case of yeasts. On minimally processed vegetables, 2-methyl-1-butanol and 3-methyl-1-butanol were found during storage of cut iceberg lettuce and washed mini-florets cut from broccoli heads (Hansen et al., 1992; Smyth et al., 1998). No microbiological counts however were given in these reports. Another alcohol was reported by Alasalvar et al. (1999) who detected 1-propanol during storage of unprocessed carrots inside paper bags after 14 days at 5 °C and after 4 days at 25 and 35 °C. The authors observed that, as the product started to visually deteriorate, an alcoholic odor was observed. They also stated that 1-propanol could be produced by micro-organisms although no microbiological analyses were performed.

The involvement of microbiological activity in off-odour production during storage of minimally processed vegetables was also proposed by Barry-Ryan and O’Beirne (1998) who related the development of off-odours detected during storage of MA packaged fresh-cut carrot slices at 8 °C related the development of off-odours detected during storage was also proposed by Barry-Ryan and O’Beirne (1998) who performed.

The production of different acids (lactic, acetic, malic, succinic, and pyruvic acids) by lactic acid bacteria has also been described by Carlin et al. (1989) and Kakiomenou et al. (1996) during storage of minimally processed carrots. Similarly, Jacksens et al. (2003) detected organic acids during the storage of shredded mixed bell peppers and grated celery at 7 °C under MA. In both cases, the psychrotrophic count exceeded 8 log cfu/g and was dominated by lactic acid bacteria (7.0–8.0 log cfu/g). In the case of mixed bell peppers, yeasts also reached high counts (5.0–5.5 log cfu/g).

From the previous paragraphs, it can be seen that the detection of off-odours is often accompanied by a bacterial count exceeding 8 log cfu/g or a yeast count exceeding 5 log cfu/g, which is similar to the counts reported when visual defects become obvious (see Section 4.1). It should however be mentioned that these counts do not necessarily cause off-odours, and neither do they necessarily result in visual defects. Giménez et al. (2003) found during storage of minimally processed artichoke stored under air conditions at 4 °C that the typical odor of artichoke was preserved during the whole storage period (15 days). The psychrotrophic count on day 15 however reached 8.8 log cfu/g.

4.3. Texture breakdown

The texture of plant products is determined by cell-wall structure and internal pressure within the cells (turgor) (Shewfelt, 1990). Pectins play an important role in this structure by providing firmness and intercellular adhesion (Kunzek et al., 1999; Wakabayashi, 2000; Ridley et al., 2001). Damaging of cells can result in the release of enzymes that degrade cell-wall polysaccharides, primarily pectins (Shewfelt, 1990). This is also the case in minimally processed vegetables due to cut surfaces (King and Bolin, 1989).

Low O₂ or high CO₂ concentrations exceeding the limits of tolerance can lead to textural breakdown in minimally processed tissues (Giménez et al., 2003; Allende et al., 2004). Giménez et al. (2003) have furthermore established that O₂ concentrations present in air result in faster texture breakdown compared to appropriate MA conditions. Besides being associated with higher respiration rates, the faster texture breakdown observed in minimally processed vegetables might also be the result of higher aerobic psychrotrophic counts. Different micro-organisms are able to produce pectinolytic enzymes which could influence textural changes in minimally processed vegetables by degrading the middle lamella and the primary cell wall. The production of pectinolytic enzymes has been reported for different species of bacteria (Juven et al., 1985; Membré and Burlot, 1994; Membré et al., 1995; Fraaije et al., 1997; Liao et al., 1997). The most frequently isolated pectinolytic bacteria regarding minimally processed vegetables are species of Erwinia and Pseudomonas. A range of pectinolytic enzymes can be produced microbiologically: pectin lyases, pectate lyase, polygalacturonase and pectin methyl esterases (Membré and Burlot, 1994; Fraaije et al., 1997; Liao et al., 1997). Besides gram-negative bacteria, L. mesenteroides, being gram-positive and belonging to the typical bacterial population of minimally processed vegetables (see Section 2.2) has been reported to have pectinolytic activity (Juven et al., 1985). Pectin degradation during fermentation processes brought about by pectinolytic strains of L. mesenteroides might enrich the medium with breakdown products that can be utilized by other lactic acid bacteria. Such...
activity could also release nutrients in the case of spoilage of vegetables. Besides the slime production and acidity that was found by Robbs et al. (1996a) on cut celery segments, \textit{L. mesenteroides} might also have been responsible for soft rot. Next to bacteria, a whole range of yeasts have been reported to produce pectinolytic enzymes, mainly endopolygalacturonases (Blanco et al., 1999). The pectinolytic activity of yeasts has mainly been studied in relation to industrial fermentation processes (Blanco et al., 1999). However, some pectinolytic yeasts (\textit{Trichosporon} sp.) and moulds (\textit{Mucor} sp. and \textit{Sclerotinia sclerotiorum}) have been isolated from shredded carrots (Carlin et al., 1989; Babic et al., 1992). Besides pectins, cellulose can be degraded by some species of \textit{Erwinia} and \textit{Pseudomonas}, \textit{Chryseomonas luteola} and \textit{Trichosporon} spp. through production of cellulases (Fleet, 1992; Laurent et al., 2000).

There seems to be inconsistency in the microbiological counts necessary to cause textural decay. On the one hand, when celery segments previously stored at 2°C for 14 days and subsequently at 5°C for 11 days were microbiologically analysed, bacterial populations of 7.0–7.7 log cfu/g were found with pectinolytic \textit{Pseudomonas} sp. predominating (Robbs et al., 1996a). This was accompanied by soft or macerated tissue. On the other hand, Robbs et al. (1996b) found after 28 days storage at 5°C of fresh-cut celery, bacterial populations of 7.1 log cfu/g in the absence of textural or colour spoilage, despite the predominance of pectinolytic \textit{Pseudomonas} spp. Carlin et al. (1989) found that, although the aerobic mesophilic count was 8.7 log cfu/g, isolated pectinolytic bacteria on ready-to-use grated carrots, stored for 14 days at 10°C, had no influence on spoilage, and Howard et al. (1994) found that during storage of packaged diced onions at 2°C, no differences in texture occurred between two batches. Total aerobic counts at the end of the storage period were 5.8 log cfu/g in the first batch and 7.1 log cfu/g in the second batch. In fresh-cut spinach, stored in gas-permeable bags at 10°C during 12 days, texture, measured by means of a shear compression cell, decreased significantly between days 2 and 5 (Babic et al., 1996). At that same time, the psychrotrophic count, dominated by \textit{P. fluorescens}, exceeded 8 log cfu/g. It seems that, as with the appearance of visual defects and off-odours, many different factors have an influence on texture breakdown, and microbiological activity is only one of those factors. It is likely that a complex mixture of bacteria rather than a single pathogenic species will initiate decay, as shown in the case of cut celery (Robbs et al., 1996a), while as with most intact plant tissues, a possible loss in plant resistance to micro-organisms resulting in ‘soft-rot’ spots or other visual symptoms (as described in Section 4.1), possibly lead to unacceptable visual defects before textural breakdown can be observed. Studies combining different sensory evaluations, including texture, found that during storage experiments under air or modified atmospheres (3–5% \textit{O2}/5–10% \textit{CO2}) with different minimally processed vegetables, visual defects and/or off-odour and off-flavours determined sensory shelf life (López-Gálvez et al., 1997; Giménez et al., 2003; Jacxsens et al., 2003).

5. Conclusions

During storage of minimally processed vegetables, damage due to cutting results in increased rates of physiological activity and leakage of nutrients, which also favours growth of micro-organisms. This reviews shows that, when changes in sensory quality factors of minimally processed vegetables result in rejection of the product, microbiological counts are in most cases high (>7–8 log cfu/g). Unacceptable colour changes can happen sooner, due to enzymatic browning in browning-sensitive products. For other visual defects, high counts (>8 log cfu/g) are necessary for visual defects to be manifested and to cause texture breakdown. Exceeding this microbiological limit does not always result in occurrence of these defects as both microbiological and physiological activity play a role in spoilage of minimally processed vegetables. Many factors determine if either microbiological or physiological spoilage mechanisms will dominate. However, there is an interaction between these mechanisms, and it is important that both be considered when studying the quality of fresh produce during storage. With regards to the microbiological population of minimally processed vegetables, yeasts can also cause spoilage at specific counts which are lower than the bacterial numbers necessary to cause similar defects. Therefore, the psychrotrophic counts as well as yeast counts should be evaluated when investigating the effect of micro-organisms on sensory quality. Moreover, when evaluating microbiological counts in relation to spoilage, surface densities of bacteria and yeasts can be higher on cut surfaces or damaged surfaces than is reflected by microbiological analysis due the dilution of such highly contaminated areas by less-contaminated non-damaged surfaces.

Visual characteristics are very important for quality differentiation at purchase. Therefore, many studies on storage life of minimally processed vegetables including sensory evaluations in their experiments, have focused on visual quality or overall quality, based on appearance, although also sensory factors can play a role in the sensory shelf life. Texture degradation is observed as a sensory characteristic when a relatively large part of the textural structure becomes degraded. This is in contrast with odour or visual defects, which can originate from some small areas of the tissue. In general, information about changes in volatile and non-volatile compounds during storage in relation to off-odours or off-flavours is scarce and more research is needed in this area. In many cases, the micro-organisms responsible for the presence of off-odours and off-flavours have not been fully identified (Whitfield, 1998). Better knowledge of micro-organisms involved in spoilage and the metabolites associated with spoilage is needed to develop microbiological and chemical methods for evaluation of quality and shelf life (Gram et al., 2002). In addition, correlations between microbiological counts, chemical changes and sensory data are needed (Dainty, 1996). Besides the direct effect of microbiological activity on flavour quality, interaction with physiological and microbiological mechanisms during storage of commodities susceptible to microbiological invasion can occur. Research on other commodities such as strawberry fruit indicates that micro-organisms can produce metabolites which can physiologically
be converted by the fruit themselves (Yu et al., 2000; Ragaert et al., 2006b). To our knowledge, no research on this interaction has been performed in the area of minimally processed vegetables.

References


