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Review

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

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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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Contents

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

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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 (Magnuson 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; Everis, 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; Everis, 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 O_2 and higher CO_2 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% O_2 and 21% O_2 at 8 °C for both *Pseudomonas* spp. and *Enterobacteriaceae*. The effect of increasing CO_2 concentration on the growth rate was not consistent. In the case of *Enterobacteriaceae*, significant decreases were found with 20% CO_2 while in the case of *Pseudomonas* spp., significant differences in growth rate could be observed from 5% CO_2. O_2 concentrations below 1.5% and CO_2

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 O_2 levels and high CO_2 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 O_2 concentrations (80–90%), in combination with moderate CO_2 concentrations (10–20%) has been investigated as an alternative to inhibit micro-organisms using modified atmospheres without a negative impact on physiology processes (CO_2-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 O_2 concentrations seemed to alleviate injuries caused by high CO_2 concentrations (15–25% CO_2) (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 (RH_{opt}) prevent moisture loss (if $RH < RH_{opt}$) or mould growth (if $RH > RH_{opt}$) (Zagory and Kader, 1988). RH_{opt} 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 Artes, 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 haloacetic 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 dis-

eases, 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 ammonium 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, dicaffeoyl 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% O_2, 20% CO_2, balance N_2) 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 discolouration 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 O_2 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). CO_2 concentrations were between 30 and 35%, while O_2 concentrations were between 2.5 and 5.0%. Samples having undergone less stressful conditions during processing (for instance, where razor blades were used with consequently 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 CO_2 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 O_2 and high CO_2 levels are dependent on different factors.

With regards to these limits of tolerance, O_2 concentrations that are too low or CO_2 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 packages leading to high CO_2 concentrations (>25%) resulted in an unacceptable visual quality with a corresponding psychrotrophic count between 6 and 7 log cfu/g. The high CO_2 concentration resulted in physiological browning and necrosis (CO_2 injury). Such high CO_2 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 O_2 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 O_2 and/or high CO_2 concentrations on production of fermentative metabolites in combination with microbiological analysis. López-Gálvez et al. (1997) correlated increases in CO_2 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. O_2 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 O_2 and 0.6–0.8 kPa O_2 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 CO_2 (10–23%) and/or low O_2 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 Jacxsens 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/cm^2 in the case of bacteria and 5.0 log cfu/cm^2 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 miniflorets 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 odour 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, to microbiological counts. Off-odours were noted when total aerobic counts reached 8 log cfu/g. This is similar to the results of Hao et al. (1999) who detected during storage of packaged shredded carrots at 4 °C a faint sour odour on day 14, when the psychrotrophic count was 8.1 ± 0.1 log cfu/g. No unacceptable odours were detected during storage of packaged shredded broccoli where the psychrotrophic count never exceeded 8 log cfu/g during 22 days.

A relationship between type of processing and storage quality was observed by Barry-Ryan and O'Beirne (1998). Fresh-cut carrot slices having undergone less stressful conditions during processing (cut by razor blades) retained a strong fresh carrot smell during 10 days of storage, while the total aerobic count never exceeded 8 log cfu/g. At day 10, the microbiological count was 7.5 log cfu/g, while the samples processed with less sharp blades contained microbiological counts of 8.7 log cfu/g at day 10.

The development of acidity, detected as sourness, is another quality feature, and Robbs et al. (1996a) found during examination of decayed fresh-cut celery, that tissue acidity and production of liquid appeared to be linked with the presence of *L. mesenteroides*. 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, Jacxsens et al. (2003) detected organic acids during

the storage of shredded mixed bell peppers and grated celeriac 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 odour 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 O_2 or high CO_2 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 O_2 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 microorganisms 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, *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 (*Trichosporon* sp.) and moulds (*Mucor* sp. and *Sclerotina 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 *Erwinia* and *Pseudomonas*, *Chryseomonas luteola* and *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 *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 *Pseudomona*s 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 *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 microbiological attack will lead to development of decay (Robbs et al., 1996b). In addition, physiological processes and/or activity of pectinolytic 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% O$_2$/5–10% CO$_2$) 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 other 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

Adams, M.R., Hartley, A.D., Cox, L.J., 1989. Factors affecting the efficacy of washing procedures used in the production of prepared salads. Food Microbiol. 6, 69–77.

Ahvenainen, R., 1996. New approaches in improving the shelf life of minimally processed fruit and vegetables. Trends Food Sci. Technol. 7, 179–187.

Alasalvar, C., Grigor, J.M., Quantick, P.C., 1999. Method for the static headspace analysis of carrot volatiles. Food Chem. 65, 391–397.

Allende, A., Artes, F., 2003. Combined ultraviolet-C and modified atmosphere packaging treatments for reducing microbial growth of fresh processed lettuce. Lebensm.-Wiss. u-Technol. 36, 779–786.

Allende, A., Luo, Y.G., Mcevoy, J.L., Artes, F., Wang, C.Y., 2004. Microbial and quality changes in minimally processed baby spinach leaves stored under super atmospheric oxygen and modified atmosphere conditions. Postharvest Biol. Technol. 33, 51–59.

Amanatidou, A., Smid, E.J., Gorris, L.G.M., 1999. Effect of elevated oxygen and carbon dioxide on the surface growth of vegetable-associated microorganisms. J. Appl. Microbiol. 86, 429–438.

Anonymous, 2005. Purchase department fruit and vegetables Delhaize Group N.V.

Babic, I., Hilbert, G., Nguyen-the, C., Guiraud, J., 1992. The yeast flora of stored ready-to-use carrots and their role in spoilage. Int. J. Food Sci. Technol. 27, 473–484.

Babic, I., Roy, S., Watada, A.E., Wergin, W.P., 1996. Changes in microbial populations on fresh cut spinach. Int. J. Food Microbiol. 31, 107–119.

Barriga, M.I., Trachy, G., Willemot, C., Simard, R.E., 1991. Microbial changes in shredded iceberg lettuce stored under controlled atmospheres. J. Food Sci. 56, 1586–1588, 1599.

Barry-Ryan, C., O'Beirne, D., 1998. Quality and shelf-life of fresh cut carrot slices as affected by slicing method. J. Food Sci. 63, 851–856.

Barry-Ryan, C., O'Beirne, D., 2000. Effects of peeling methods on the quality of ready-to-use carrot slices. Int. J. Food Sci. Technol. 35, 243–254.

Bennik, M.H.J., Peppelenbos, H.W., Nguyen-the, C., Carlin, F., Smid, E.J., Gorris, L.G.M., 1996. Microbiology of minimally processed, modified-atmosphere packaged chicory endive. Postharvest Biol. Technol. 9, 209–221.

Bennik, M.H.J., Vorstman, W., Smid, E.J., Gorris, L.G.M., 1998. The influence of oxygen and carbon dioxide on the growth of prevalent *Enterobacteriaceae* and *Pseudomonas* species isolated from fresh and controlled-atmosphere-stored vegetables. Food Microbiol. 15, 459–469.

Blanco, P., Sieiro, C., Villa, T.G., 1999. Production of pectic enzymes in yeasts. FEMS Microbiol. Lett. 175, 1–9.

Boschetti, A., Biasioli, F., Van Opbergen, M., Warneke, C., Jordan, A., Holzinger, R., Prazeller, P., Karl, T., Hansel, A., Lindinger, W., Iannotta, S., 1999. PTR-MS real time monitoring of the emission of volatile organic compounds during postharvest aging of berryfruit. Postharvest Biol. Technol. 17, 143–151.

Brackett, R.E., 1999. Incidence, contributing factors, and control of bacterial pathogens in produce. Postharvest Biol. Technol. 15, 305–311.

Brinkman, J.H.M., 2002. Zintuigen en waarneming. In: Brinkman. Proeven van succes. Houten, Keesing Noordervliet.

Brocklehurst, T.F., Lund, B.M., 1981. Properties of Pseudomonads causing spoilage of vegetables stored at low-temperature. J. Appl. Bacteriol. 50, 259–266.

Cadwallader, K.R., 2005. Flavor and volatile metabolism in produce. In: Lamikanra, O., Imam, S., Ukuku, D. (Eds.), Produce Degradation: Pathways and Prevention. Taylor & Francis Group, London.

Carlin, F., Nguyen-the, C., Cudennec, P., Reich, M., 1989. Microbiological spoilage of fresh, ready-to-use grated carrots. Sci. Aliments 9, 371–386.

Chang, C.Y., Hsieh, Y.H., Hsu, S.S., Hu, P.Y., Wang, K.H., 2000. The formation of disinfection by-products in water treated with chlorine dioxide. J. Hazard. Mater. 79, 89–102.

Cliffe-Byrnes, V., O'Beirne, D., 2005. The effects of cultivar and physiological age on quality and shelf-life of coleslaw mix packaged in modified atmospheres. Int. J. Food Sci. Technol. 40, 165–175.

Dainty, R.H., 1996. Chemical/biochemical detection of spoilage. Int. J. Food Microbiol. 33, 19–33.

Day, B.P.F., 1993. Fruit and vegetables. In: Parry, R.T. (Ed.), Principles and Applications of Modified Atmosphere Packaging of Foods. Blackie Academic & Professional, London.

Devlieghere, F., Vermeulen, A., Debevere, J., 2004. Chitosan: antimicrobial activity, interactions with food components and applicability as a coating on fruit and vegetables. Food Microbiol. 21, 703–714.

Elmore, J.S., Erbahadir, M.A., Mottram, D.S., 1997. Comparison of dynamic headspace concentration on Tenax with solid phase microextraction for the analysis of aroma volatiles. J. Agric. Food Chem. 45, 2638–2641.

Emmambux, N.M., Minnaar, A., 2003. The effect of edible coatings and polymeric packaging films on the quality of minimally processed carrots. J. Sci. Food Agric. 83, 1065–1071.

Everis, L., 2004. Risks of pathogens in ready-to-eat fruits, vegetables, and salads through the production process. Review No. 44. Gloucestershire, Campden & Chorleywood Food Research Association Group.

Ferreres, F., Gil, M.I., Castaner, M., Tomas-Barberan, F.A., 1997. Phenolic metabolites in red pigmented lettuce (*Lactuca sativa*). Changes with minimal processing and cold storage. J. Agric. Food Chem. 45, 4249–4254.

Fleet, G.H., 1992. Spoilage yeasts. Crit. Rev. Biotechnol. 12, 1–44.

Forney, C.F., Kalt, W., Jordan, M.A., 2000. The composition of strawberry aroma is influenced by cultivar, maturity, and storage. Hortscience 35, 1022–1026.

Fraaije, B.A., Bosveld, M., Vandenbulk, R.W., Rombouts, F.M., 1997. Analysis of conductance responses during depolymerization of pectate by soft rot *Erwinia* spp. and other pectolytic bacteria isolated from potato tubers. J. Appl. Microbiol. 83, 17–24.

Francis, G.A., Thomas, C., O'Beirne, D., 1999. The microbiological safety of minimally processed vegetables. Int. J. Food Sci. Technol. 34, 1–22.

García-Gimeno, R.M., Zurera-Cosano, G., 1997. Determination of ready-to-eat vegetable salad shelf life. Int. J. Food Microbiol. 36, 31–38.

Garg, N., Churey, J.J., Splittstoesser, D.F., 1990. Effect of processing conditions on the microflora of fresh-cut vegetables. J. Food Prot. 53, 701–703.

Gil, M.I., Castaner, M., Ferreres, F., Artes, F., Tomas-Barberan, F.A., 1998. Modified-atmosphere packaging of minimally processed "*Lollo rosso*" (*Lactuca sativa*)—phenolic metabolites and quality changes. Z. Lebensm. Unters. Forsch. 206, 350–354.

Giménez, M., Olarte, C., Sanz, S., Lomas, C., Echávarri, J.F., Ayala, F., 2003. Relation between spoilage and microbiological quality in minimally processed artichoke packaged with different films. Food Microbiol. 20, 231–242.

Gomez-Lopez, V.M., Devlieghere, F., Bonduelle, V., Debevere, J., 2005. Intense light pulses decontamination of minimally processed vegetables and their shelf-life. Int. J. Food Microbiol. 103, 79–89.

Gram, L., Ravn, L., Rasch, M., Bruhn, J.B., Christensen, A.B., Givskov, M., 2002. Food spoilage—interactions between food spoilage bacteria. Int. J. Food Microbiol. 78, 79–97.

Hagenmaier, R.D., Baker, R.A., 1998. A survey of the microbial population and ethanol content of bagged salad. J. Food Prot. 61, 357–359.

Han, Y., Sherman, D.M., Linton, R.H., Nielsen, S.S., Nelson, P.E., 2000. The effects of washing and chlorine dioxide gas on survival and attachment of *Escherichia coli* O157:H7 to green pepper surfaces. Food Microbiol. 17, 521–533.

Hansen, M., Buttery, R.G., Stern, D.J., Cantwell, M.I., Ling, L.C., 1992. Broccoli storage under low-oxygen atmosphere: identification of higher boiling volatiles. J. Agric. Food Chem. 40, 850–852.

Hao, Y.Y., Brackett, R.E., Beuchat, L.R., Doyle, M.P., 1999. Microbiological quality and production of botulinal toxin in film-packaged broccoli, carrots, and green beans. J. Food Prot. 62, 499–508.

Heimdal, H., Kühn, B.F., Poll, L., Larsen, L.M., 1995. Biochemical changes and sensory quality of shredded and MA-packaged iceberg lettuce. J. Food Sci. 60, 1265–1268.

Holt, R.U., 2001. Mechanisms effecting analysis of volatile flavour components by solid-phase microextraction and gas chromatography. J. Chromatogr. A 937, 107–114.

Hong, S.I., Kim, D., 2004. The effect of packaging treatment on the storage quality of minimally processed bunched onions. Int. J. Food Sci. Technol. 39, 1033–1041.

Howard, L.R., Yoo, K.S., Pike, L.M., Miller, G.H., 1994. Quality changes in diced onions stored in film packages. J. Food Sci. 59, 110–112, 117.

Izumi, H., 1999. Electrolyzed water as a disinfectant for fresh-cut vegetables. J. Food Sci. 64, 536–539.

Jacques, M.A., Morris, C.E., 1995. Bacterial population-dynamics and decay on leaves of different ages of ready-to-use broad-leaved endive. Int. J. Food Sci. Technol. 30, 221–236.

Jacxsens, L., Devlieghere, F., Debevere, J., 2002. Temperature dependence of shelf-life as affected by microbial proliferation and sensory quality of equilibrium modified atmosphere packaged fresh produce. Postharvest Biol. Technol. 26, 59–73.

Jacxsens, L., Devlieghere, F., Ragaert, P., Vanneste, E., Debevere, J., 2003. Relation between microbiological quality, metabolite production and sensory quality of equilibrium modified atmosphere packaged fresh-cut produce. Int. J. Food Microbiol. 83, 263–280.

Jacxsens, L., Devlieghere, F., Van der Steen, C., Debevere, J., 2001. Effect of high oxygen modified atmosphere packaging on microbial growth and sensorial qualities of fresh-cut produce. Int. J. Food Microbiol. 71, 197–210.

Juven, B.J., Lindner, P., Weisslowicz, H., 1985. Pectin degradation in plant-material by *Leuconostoc mesenteroides*. J. Appl. Bacteriol. 58, 533–538.

Kader, A.A., Mitcham, B., 1997. Standardization of quality. Fresh-cut product: maintaining quality and safety. Postharvest Horticult Ser 10, 5.1–5.3.

Kakiomenou, K., Tassou, C., Nychas, G.J., 1996. Microbiological, physico-chemical and organoleptic changes of shredded carrots stored under modified storage. Int. J. Food Sci. Technol. 31, 359–366.

Kang, H.M., Saltveit, M.E., 2002. Antioxidant capacity of lettuce leaf tissue increases after wounding. J. Agric. Food Chem. 50, 7536–7541.

Kelly, W.J., Davey, G.P., Ward, L.J.H., 1998. Characterization of Lactococci isolated from minimally processed fresh fruit and vegetables. Int. J. Food Microbiol. 45, 85–92.

Kim, J.G., Yousef, A.E., Dave, S., 1999. Application of ozone for enhancing the microbiological safety and quality of foods: a review. J. Food Prot. 62, 1071–1087.

King, A.D., Bolin, H.R., 1989. Physiological and microbiological storage stability of minimally processed fruits and vegetables. Food Technol. 43, 132–135, 139.

King, A.D., Magnuson, J.A., Torok, T., Goodman, N., 1991. Microbial flora and storage quality of partially processed lettuce. J. Food Sci. 56, 459–461.

Klein, B.P., 1987. Nutritional consequences of minimal processing of fruits and vegetables. J. Food Qual. 10, 179–193.

Kunzek, H., Kabbert, R., Gloyna, D., 1999. Aspects of material science in food processing: changes in plant cell walls of fruits and vegetables. Z. Lebensm. Unters. Forsch. 208, 233–250.

Lamikanra, O., 2005. Mechanical injury of fresh produce. In: Lamikanra, O., Imam, S., Ukuku, D. (Eds.), Produce Degradation: Pathways and Prevention. Taylor & Francis Group, London.

Laurent, P., Buchon, L., Guespin-Michel, J.F., Orange, N., 2000. Production of pectate lyases and cellulases by *Chryseomonas luteola* strain MFCL0 depends on the growth temperature and the nature of the culture medium: evidence for two critical temperatures. Appl. Environ. Microbiol. 66, 1538–1543.

Laurila, E., Ahvenainen, R., 2002. Minimal processing in practice: fresh fruits and vegetables. In: Ohlsson, T., Bengtsson, N. (Eds.), Minimal Processing Technologies in the Food Industry. Woodhead Publishing Limited, Cambridge.

Laycock, M.V., Hildebrand, P.D., Thibault, P., Walter, J.A., Wright, J.L.C., 1991. Viscosin, a potent peptidolipid biosurfactant and phytopathogenic mediator produced by a pectolytic strain of *Pseudomonas fluorescens*. J. Agric. Food Chem. 39, 483–489.

Legnani, P.P., Leoni, E., 2004. Effect of processing and storage conditions on the microbiological quality of minimally processed vegetables. Int. J. Food Sci. Technol. 39, 1061–1068.

Li, Y., Brackett, R.E., Chen, J., Beuchat, L.R., 2002. Mild heat treatment of lettuce enhances growth of *Listeria monocytogenes* during subsequent storage at 5 degrees C or 15 degrees C. J. Appl. Microbiol. 92, 269–275.

Li, Y., Brackett, R.E., Shewfelt, R.L., Beuchat, L.R., 2001. Changes in appearance and natural microflora on iceberg lettuce treated in warm, chlorinated water and then stored at refrigeration temperature. Food Microbiol. 18, 299–308.

Liao, C.H., Sullivan, J., Grady, J., Wong, L.J.C., 1997. Biochemical characterization of pectate lyases produced by fluorescent pseudomonads associated with spoilage of fresh fruits and vegetables. J. Appl. Microbiol. 83, 10–16.

López-Gálvez, G., Peiser, G., Nie, X.L., Cantwell, M., 1997. Quality changes in packaged salad products during storage. Z. Lebensm. Unters. Forsch. 205, 64–72.

López-Gálvez, G., Saltveit, M., Cantwell, M., 1996. The visual quality of minimally processed lettuces stored in air or controlled atmosphere with emphasis on romaine and iceberg types. Postharvest Biol. Technol. 8, 179–190.

Lund, B.M., 1992. Ecosystems in vegetable foods. J. Appl. Bacteriol. 73, S115–S126.

Magnuson, J.A., King, A.D., Torok, T., 1990. Microflora of partially processed lettuce. Appl. Environ. Microbiol. 56, 3851–3854.

Martinez, M.V., Whitaker, J.R., 1995. The biochemistry and control of enzymatic browning. Trends Food Sci. Technol. 6, 195–200.

Membré, J.M., Burlot, P.M., 1994. Effects of temperature, pH, and NaCl on growth and pectinolytic activity of Pseudomonas marginalis. Appl. Environ. Microbiol. 60, 2017–2022.

Membré, J.M., Goubet, D., Kubaczka, M., 1995. Influence of salad constituents on growth of Pseudomonas marginalis—a predictive microbiology approach. J. Appl. Bacteriol. 79, 603–608.

Mercier, J., Lindow, S.E., 2000. Role of leaf surface sugars in colonization of plants by bacterial epiphytes. Appl. Environ. Microbiol. 66, 369–374.

Moss, M.O., 1999. Spoilage problems/problems caused by fungi. In: Robinson, R.K., Batt, C.A., Patel, P.D. (Eds.), Encyclopedia of Food Microbiology. Academic Press, London.

Nguyen-the, C., Carlin, F., 1994. The microbiology of minimally processed fresh fruits and vegetables. Crit. Rev. Food Sci. Nutr. 34, 371–401.

Nguyen-the, C., Prunier, J.P., 1989. Involvement of Pseudomonads in deterioration of ready-to-use salads. Int. J. Food Sci. Technol. 24, 47–58.

Nieuwenhuijsen, M.J., Toledano, M.B., Elliot, P., 2000. Uptake of chlorination disinfection by-products; a review and a discussion of its implications for exposure assessment in epidemiological studies. J. Expo. Anal. Environ. Epidemiol. 10, 586–599.

Paull, R.E., 1999. Effect of temperature and relative humidity on fresh commodity quality. Postharvest Biol. Technol. 15, 263–277.

Prakash, A., Guner, A.R., Caporaso, F., Foley, D.M., 2000. Effects of low-dose gamma irradiation on the shelf life and quality characteristics of cut romaine lettuce packaged under modified atmosphere. J. Food. Sci. 65, 549–553.

Ragaert, P., Devlieghere, F., Devuyst, E., Dewulf, J., Van Langenhove, H., Debevere, J., 2006a. Volatile metabolite production of spoilage micro-organisms on a mixed-lettuce agar during storage at 7 °C in air and low oxygen atmosphere. Int. J. Food Microbiol. 112, 162–170.

Ragaert, P., Devlieghere, F., Loos, S., Dewulf, J., Van Langenhove, H., Foubert, I., Vanrolleghem, P.A., Debevere, J., 2006b. Role of yeast proliferation in the quality degradation of strawberries during refrigerated storage. Int. J. Food Microbiol. 108, 42–50.

Ragaert, P., Devlieghere, F., Ryckeboer, J., Coosemans, J., Debevere, J., 2004a. Nieuwe decontaminatietechnieken voor groenten en fruit. VMT 37, 18–20.

Ragaert, P., Verbeke, W., Devlieghere, F., Debevere, J., 2004b. Consumer perception and choice of minimally processed vegetables and packaged fruits. Food Qual. Prefer. 15, 259–270.

Ridley, B.L., O'Neill, M.A., Mohnen, D.A., 2001. Pectins: structure, biosynthesis, and oligogalacturonide-related signaling. Phytochemistry 57, 929–967.

Robbs, P.G., Bartz, J.A., Mcfie, G., Hodge, N.C., 1996a. Causes of decay of fresh-cut celery. J. Food Sci. 61, 444–448.

Robbs, P.G., Bartz, J.A., Sargent, S.A., Mcfie, G., Hodge, N.C., 1996b. Potential inoculum sources for decay of fresh-cut celery. J. Food Sci. 61, 449–452, 455.

Roura, S.I., Davidovich, L.A., del Valle, C.E., 2000. Quality loss in minimally processed swiss chard related to amount of damaged area. Lebensm.-Wiss. u-Technol 33, 53–59.

Saltveit, M.E., 1999. Effect of ethylene on quality of fresh fruits and vegetables. Postharvest Biol. Technol. 15, 279–292.

Schaller, E., Bosset, J.O., Escher, F., 1998. 'Electronic noses' and their application to food. Lebensm.-Wiss. u-Technol. 31, 305–316.

Schlimme, D.V., Rooney, M.L., 1994. Packaging of minimally processed fruits and vegetables. In: Wiley, R.C. (Ed.), Minimally Processed Refrigerated Fruits & Vegetables. Chapman & Hall, London.

Seymour, I.J., Appleton, H., 2001. Foodborne viruses and fresh produce. J. Appl. Microbiol. 91, 759–773.

Seymour, I.J., Burfoot, D., Smith, R.L., Cox, L.A., Lockwood, A., 2002. Ultrasound decontamination of minimally processed fruits and vegetables. Int. J. Food Sci. Technol. 37, 547–557.

Shewfelt, R.L., 1990. Quality of fruits and vegetables. Food Technol. 44, 99–106.

Singh, N., Singh, R.K., Bhunia, A.K., Stroshine, R.L., 2002. Efficacy of chlorine dioxide, ozone, and thyme essential oil or a sequential washing in killing *Escherichia coli* O157:H7 on lettuce and Baby carrots. Lebensm.-Wiss. u-Technol. 35, 720–729.

Sivapalasingam, S., Friedman, C.R., Cohen, L., Tauxe, R.V., 2004. Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. J. Food Prot. 67, 2342–2353.

Smyth, A.B., Song, J., Cameron, A.C., 1998. Modified atmosphere packaged cut iceberg lettuce: effect of temperature and O_2 partial pressure on respiration and quality. J. Agric. Food Chem. 46, 4556–4562.

Smyth, A.B., Talasila, P.C., Cameron, A.C., 1999. An ethanol biosensor can detect low-oxygen injury in modified atmosphere packages of fresh-cut produce. Postharvest Biol. Technol. 15, 127–134.

Snowdon, A.L., 1990. Nature of post-harvest deterioration. In: Snowdon, A.L. (Ed.), A Colour Atlas of Post-harvest Diseases & Disorders of Fruits and Vegetables: General Introduction & Fruits, vol. 1. Wolfe Scientific, London.

Surjadinata, B.B., Cisneros-Zevallos, L., 2003. Modeling wound-induced respiration of fresh-cut carrots (*Daucus carota* L.). J. Food Sci. 68, 2735–2740.

Takeuchi, K., Frank, J.F., 2001. Quantitative determination of the role of lettuce leaf structures in protecting *Escherichia coli* O157:H7 from chlorine disinfection. J. Food Prot. 64, 147–151.

Toivonen, P.M.A., 1997. Non-ethylene, non-respiratory volatiles in harvested fruits and vegetables: their occurrence, biological activity and control. Postharvest Biol. Technol. 12, 109–125.

Tomás-Barberán, F., Espín, J.C., 2001. Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. J. Sci. Food Agric. 81, 853–876.

Tournas, V.H., 2005. Moulds and yeasts in fresh and minimally processed vegetables, and sprouts. Int. J. Food Microbiol. 99, 71–77.

Vankerschaver, K., Willocx, F., Smout, C., Hendrickx, M., Tobback, P., 1996. The influence of temperature and gas mixtures on the growth of the intrinsic micro-organisms on cut endive: predictive versus actual growth. Food Microbiol. 13, 427–440.

Wakabayashi, K., 2000. Changes in cell wall polysaccharides during fruit ripening. J. Plant Res. 113, 231–237.

Watada, A.E., Ko, N.P., Minott, D.A., 1996. Factors affecting quality of fresh-cut horticultural products. Postharvest Biol. Technol. 9, 115–125.

Watada, A.E., Qi, L., 1999. Quality of fresh-cut produce. Postharvest Biol. Technol. 15, 201–205.

Whitfield, F.B., 1998. Microbiology of food taints. Int. J. Food Sci. Technol. 33, 31–51.

Wilkes, J.G., Conte, E.D., Kim, Y., Holcomb, M., Sutherland, J.B., Miller, D.W., 2000. Sample preparation for the analysis of flavors and off-flavors in foods. J. Chromatogr. A 880, 3–33.

Yu, K.S., Hamilton-Kemp, T.R., Archbold, D.D., Collins, R.W., Newman, M.C., 2000. Volatile compounds from *Escherichia coli* O157:H7 and their absorption by strawberry fruit. J. Agric. Food Chem. 48, 413–417.

Zagory, D., 1999. Effects of post-processing handling and packaging on microbial populations. Postharvest Biol. Technol. 15, 313–321.

Zagory, D., Kader, A.A., 1988. Modified atmosphere packaging of fresh produce. Food Technol. 42, 70–77.