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Predictive modelling for packaging design: equilibrium modified atmosphere packages of fresh-cut vegetables subjected to a simulated distribution chain

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Abstract

The impact of temperature fluctuations in a simulated cold distribution chain, typical of commercial practice, was investigated on both the microbial and sensorial quality of equilibrium modified atmosphere (EMA) packaged minimally processed vegetables. The internal O_2 concentration of the designed packages could be predicted for the different steps of the simulated distribution chain by applying an integrated mathematical system. The internal atmosphere in the packages remained in its aerobic range during storage in the chain due to the application of high permeable packaging films for O_2 and CO_2. Spoilage microorganisms were proliferating fast on minimally processed bell peppers and lettuce. Yeasts showed to be the shelf-life limiting group. Visual properties limited the sensorial shelf-life. *Listeria monocytogenes* was able to multiply on cucumber slices, survived on minimally processed lettuce and decreased in number on bell peppers due to the combination of low pH and refrigeration. *Aeromonas caviae* was multiplying on both cucumber slices and mixed lettuce, but was as well inhibited by the low pH of bell peppers. Storage temperature control was found to be of paramount importance for the microbial (spoilage and safety) and sensorial quality evaluation of EMA-packaged minimally processed vegetables.

Keywords: Minimally processed vegetables; Spoilage; Safety; Distribution chain; Shelf-life

1. Introduction

In recent years, new food packaging concepts have been developed to respond on consumption trends towards mildly preserved, fresh convenient food products. Packaging fresh-cut vegetables under an equilibrium modified atmosphere (EMA) is one of the new applied food packaging technologies offering a prolonged shelf-life of respiring products by suppression of their respiration rate (Day, 1996). EMA generation is still the major goal of polymer permeability (for O_2 and CO_2) modification. By matching film permeability for O_2 and CO_2 to the respiration rate of the packaged fresh-cut produce, an equilibrium modified atmosphere can be established inside the package consisting out of a decreased O_2 concentration and increased CO_2 concentration (Day, 1996). The use of low oxygen concentration (1–5%) and high carbon dioxide concentration (5–10%) (balance N_2), in com-

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bination with storage at refrigeration temperatures (≤ 7 °C), is proposed by many researchers as optimal storage conditions for fresh-cut vegetables to maintain the sensorial as well as the microbial quality (Kader et al., 1989; Philips, 1996). The enzymatic browning reaction on cut surfaces is inhibited and the general structure of the plant tissue sustains longer in its typical turgoricidy and crispness resulting in a better protection for microbial invasion (Kader et al., 1989; Bennik, 1997). Packaged fruits and vegetables are usually exposed to varying surrounding temperatures during handling, transportation, storage and marketing. Changes in the environmental temperature creates a specific problem in EMA design because the respiration rate is more influenced by temperature changes than is the O_2 and CO_2 permeability of the permeable films used to obtain the EMAs. Due to this fact, it is difficult to maintain an optimum atmosphere inside a package when the surrounding temperature is not constant. A film that produces a favourable atmosphere at the temperature for which the package was designed for, may cause excessive accumulation of CO_2 and/or depletion of O_2 at higher temperatures caused by an increased respiration rate. This anoxic situation could lead to metabolic disorders such as fermentation with production of off-flavours (Kader et al., 1989; Silva et al., 1999). In the case of a lower storage temperature, a decreased respiratory activity will lead to an accumulation of O_2 above the optimal 3% resulting in a less effective EMA package.

An integrated mathematical system was previously developed to design EMA packages for fresh-cut produce to be stored at a constant temperature of 7 °C but were subjected to varying temperatures between 2 and 15 °C (Jacxsens et al., 2000a). In this integrated mathematical system, the effect of temperature on produce respiration and film permeability was described by an Arrhenius type of equation while the effect of O_2 and CO_2 on respiration rate by a Michaelis–Menten kinetic. Prediction and validation of the integrated approach showed that optimum EMA conditions could be generated between 2 and 10 °C. Above 10 °C, an overestimation of the internal O_2 concentrations occurred probably because of the unconsidered O_2 consumption of higher microbial activity at these examples of temperature abuse. Anoxic conditions started from 12 °C (Jacxsens et al., 2000a,b).

Storage temperature is found to be of paramount importance for the microbial and visual quality evolution of minimally processed vegetables. Knowledge about the time–temperature conditions in the cold chain of ready-to-eat vegetables is necessary to determine the influence of the actual cold chain on the quality loss and the shelf-life of these products (Willocx, 1995). In Belgium, there is no specific temperature control legislation for minimally processed vegetables and are therefore categorised with all refrigerated products. For these products, the temperature must be kept at 7 °C at maximum with a tolerance up to 10 °C in the warmest spot (Anonymous, 1982). In the French legislation for 'légumes de la 4e gamme', minimally processed vegetables must be stored at maximum 4 °C (Anonymous, 1988). Time–temperature conditions at the producer are an essential Critical Control Point in a HACCP system and must be monitored. Air temperature during sorting, grading and preparation must be lower than 12 °C, while during washing, cutting and packaging, air temperature should be maintained between 4 and 6 °C. From the moment of cutting of the produce, product temperature must be controlled at a maximum of 4 °C (Willocx, 1995). Generally, refrigerated vehicles control the temperature of the air supplied to the cargo space and monitor constantly temperature of the air returning to the refrigeration units. The main problem in maintaining a required temperature control (i.e. the temperature printed on the label of the product or the legal temperature) during transport arises from the number of door openings and the amount of times doors may stay open while orders are prepared and delivered (Willocx, 1995). Rapid temperature increase of products can occur on transfer from the temperature-controlled vehicles to ambient conditions during unloading at the distributor. Moreover, the temperature performance and control in chilled display cabinets in supermarkets is rather poor. Temperatures above 7 °C are common and depend on the place (top, middle or bottom) in the chilled counter (Tolstoy, 1991; Willocx, 1995). Unlike refrigeration systems in the rest of the cold chain, domestic refrigerators are not subjected to temperature monitoring or legislative control. Temperature abuse, such as storage at ambient temperature and improper cooling ($T > 7$ °C), has been identified as the major contributory factor in outbreaks of foodborne diseases (Bryan, 1988; Brackett, 1999). Al-

though mishandling may occur at any stage of the food chain, epidemiological data show that mishandling occurs most frequently during the final stages like in food service and home preparation (Hurst and Schuler, 1992; Brackett, 1999). Improper temperature management during storage in factories, distribution and display in retail shops allows the spoilage bacteria in fresh products to multiply quickly (Kaneko et al., 1999). The susceptibility of vegetable processing and storage procedures to microbial contamination and temperature abuse is well recognised (Francis et al., 1999). The possibility of contamination with psychrotrophic foodborne pathogens (*Listeria monocytogenes* and *Aeromonas caviae*) is of concern to guarantee the safety of minimally processed, packaged vegetables (Palumbo, 1986; Jacxsens et al., 1999a; Szabo et al., 2000). Given enough time, psychrotrophic pathogens can grow to high populations in packaged produce, even if proper temperatures are maintained. Moreover, high populations of pathogens can often exist in absence of obvious sensory defects (Berrang et al., 1989a,b). The microbial spoilage and safety aspects of the produce will be different in the case of anoxic conditions inside the package. A shift will be noticed towards more microaerophilic and facultative anaerobic types of spoilage microorganisms such as lactic acid bacteria and Enterobacteriaceae. Next to this, also psychrotrophic anaerobic pathogenic bacteria as *Clostridium botulinum* have to be taken in consideration during safety evaluation in the case of anoxic conditions (Brackett, 1999).

The impact of the temperature fluctuations through a simulated "worst case" distribution chain, typical of commercial practice, is investigated in this research work by following/predicting the internal gas concentration, the survival/growth of spoilage microorganisms, the survival/growth of artificially inoculated pathogenic bacteria *L. monocytogenes* and *A. caviae*, as well as the evolution of the sensorial properties within EMA-packaged mixed lettuce, shredded and mixed bell peppers and cucumber slices in order to determine their actual shelf-life.

2. Materials and methods

2.1. Simulation of the distribution chain

The simulated distribution chain was chosen as such that the maximum time–temperature (t/T)-conditions were representative for chilled distribution of fresh-cut produce in Belgium. An overview of the different steps and their time–temperature conditions in the simulated cold chain are given in Table 1. The moments of sampling are indicated in bold. At these moments, the internal gas concentration, number of spoilage/inoculated pathogenic microorganisms and the evolution of the sensorial properties were evaluated in duplicate.

To simulate this distribution chain, different refrigerated climate rooms with forced ventilation were used, of which the inside temperature was monitored

Table 1
Schematic overview of the applied time–temperature combinations to simulate a "worst case" chilling distribution chain (t = time, T = temperature)

Step in distribution chain	Time–temperature combination	Moment of sampling
Processing–packaging	**$T < 12$ °C**	moment of sampling 0
Storage at producer	**$T = 4$ °C, $t_{maximum} = 24$ h**	moment of sampling 1
Transport from producer to distribution centre	$T = 2–3$ °C, $T_{maximum} = 5$ °C, $t = 2$ h	
Storage at distribution centre	**$T = 10$ °C, $t_{maximum} = 24$ h**	moment of sampling 2
Transport from distribution centre to supermarket	$T = 2–3$ °C, $T_{maximum} = 5$ °C, $t = 2$ h	
Unloading at the supermarket and first storage	**$T = 10$ °C, $t = 1$ h, $t_{maximum} = 8$ h**	moment of sampling 3
Storage at chilled counter	**$T = 7$ °C, $t_{maximum} = 48$ h**	moment of sampling 4
Purchase by the consumer and transport at domestic refrigerator	**$T = 20$ °C, $t = 2$ h**	moment of sampling 5
Storage in domestic refrigerator	**$T = 7$ °C, $t = ?$**	moments of sampling 6, 7 and 8

The bold steps are the moments of sampling.

($T \pm 0.2$ °C). In one of the EMA packages an electronic data logger (Escort, Tech. Innovators, New Zealand) time–temperature recorder was added to allow continuous registration (every 20 min) of the produce temperature.

2.2. Design of optimal EMA package at 7 °C

The applied EMA packages for the three types of vegetables were designed to be stored at 7 °C, based on the steady-state equation of Solomos (1994):

$$RO_2 \cdot W = PO_2 \cdot A\{(O_2)_{out} - (O_2)_{in}\} \quad (1)$$

where $(O_2)_{out}$ is the O_2 concentration outside the package (%) (air = 20.9%), $(O_2)_{in}$ is the O_2 concentration inside the package (%) (optimal = 3%), A is the area of the film (m^2) = length × width × 2, W = filling weight (kg), PO_2 is the required permeability for O_2 (ml O_2/(m^2 24 h atm)) at 7 °C and 90% RH, RO_2 is the respiration rate of the packaged vegetable at 7 °C and 3% O_2, expressed as the O_2 consumption (ml O_2/(kg h)). The permeability of the packaging film for O_2 could be calculated by using Eq. (1) in order to obtain an equilibrium of 3–5% O_2 at 7 °C (Jacxsens et al., 1999b). Based on the required permeability for O_2, the packaging film with the closest oxygen transmission rate was chosen (high permeable packaging films, Hyplast, Hoogstraten, Belgium). No selection in packaging film was made based on the CO_2 permeability because the permeability of these films is very high and an equilibrium around 3–4% CO_2 is always obtained (Jacxsens et al., 1999a,b, 2000a,b). The packaging configuration and packaging film applied in this work, for the three types of fresh-cut vegetables are tabulated in Table 2.

The produce was industrially prepared in a local vegetable processing industry. Mixed lettuce is a commercially available mixture of endive (*Cichorium endivia* L.), lollo rosso and lollo bionta (*Lactuca sativa* var. *crispa* L., (red and green variety)) and radicchio (*C. intybus* var. *foliosum* L.). Chopped bell peppers are a mixture of green, red and yellow shredded bell peppers (*Capsicum annuum* L.) (0.4 × 1 cm). Cucumber (*Cucumis sativus* L.) was sliced into 0.3-cm slices in the laboratory using a Kitchen aid (Philips, Eindhoven, The Netherlands). After refrigerated transport to the laboratory, vegetables were weighed, filled in the appropriated bags and packaged under EMA. The modification of the atmosphere (3% O_2–5% CO_2–balance N_2) in the packages was performed in an active way by using a gas packaging unit (gas mixer, WITT M618-3MSO, Gasetechnik, Germany; vacuum compensation chamber, Multivac A300/42 Hagenmüller, Wolfertschwenden, Germany). Air products (Vilvoorde, Belgium) supplied the gases O_2, CO_2 and N_2 (Fresh line). After packaging, time 0 started of the simulation of the cold distribution chain (moment of sampling 0).

2.3. Challenge test

Inoculation of the vegetables was performed with a mixture of different *L. monocytogenes* strains (Scott A, ATCC 53 and LM LJ1, a strain isolated out of red and green bell peppers at the Laboratory of Food Microbiology and Food Preservation, Ghent Univer-

Table 2
Packaging configuration and applied packaging film for three types of fresh-cut vegetables in order to obtain an EMA at 7 °C

Type of vegetable	Filling weight (g)	Area of package (cm × cm)	Calculated permeability for O_2 necessary to obtain 3% O_2 at 7 °C (ml O_2/(m^2 24 h atm)) at 7 °C	Oxygen transmission rate of the applied film (ml O_2/(m^2 24 h atm)) at 7 °C (± 95% confidence)	Film thickness (μm)
Mixed shredded lettuce	250	27 × 19.5	2026	1945 ± 229	40
Mixed shredded bell peppers	150	19 × 15	2838	2897 ± 471	40
Cucumber slices	150	19 × 15	2484	2543 ± 123	50

sity, Ghent, Belgium) and a mixture of two different *A. caviae* (HG4) strains isolated at laboratory of Food Microbiology and Food Preservation, Ghent University, from fresh spinach (Aer 9) and garden sorrel (Aer 8) (Neyts et al., 2000). Each strain was individually cultured from a refrigerated slant (TSA (Oxoid, Unipath, Basing store, Hampshire, UK, CM131) for *L. monocytogenes* and nutrient agar (NB (Oxoid, CM1) with 10 g/l agar (Oxoid, L11)) for *A. caviae*) and two times 24 h consecutively subcultured in brain heart infusion (BHI, Oxoid, CM225) at 30 °C for *A. caviae* and at 37 °C for *L. monocytogenes*. The second culture was diluted in peptone saline solution (8.5 g/l NaCl + 1 g/l peptone (Oxoid, L34)) to such an extent that a contamination level of approximately 10^3 to 10^4 viable cells/g of vegetable was reached. The vegetables were sprayed with the different pathogen inocula and gently mixed to have a homogeneous distribution of the inoculated pathogens on the fresh-cut produce. The inoculated vegetables were similarly gas packaged as the non-inoculated vegetables (Table 2).

2.4. Headspace gas analysis

At each sampling point, the gaseous composition of the headspace atmosphere in two identically prepared and stored packages was analysed using a Servomex Food Packaged (Seri 1400, Crowborough, Sussex, UK). Prediction of the internal O_2 concentration was possible with the integrated mathematical system (Jacxsens et al., 2000a):

$$RO_2 \cdot W = PO_2 \cdot A\{(O_2)_{out} - (O_2)_{in}\} \quad (1)$$

$$RO_2 = RO_2^* \exp\ (-E^R O_2/RT)$$
(expressing the temperature dependence of the respiration rate) (2)

$$PO_2 = P^* O_2 \exp\ (-E^P O_2/RT)$$
(expressing the temperature dependence of the film permeability for O_2) (3)

$$RO_2 = V_{max}(O_2)_{in}/(K_M + (O_2)_{in})$$
(expressing the O_2 dependence of the respiration rate) (4)

$$V_{max} = V_1 \exp\ (V_2 T)$$
(expressing the temperature dependence of the maximum respiration rate V_{max}) (5)

where $(O_2)_{out}$ is the O_2 concentration outside the package (%) (air = 20.9%), $(O_2)_{in}$ is the O_2 concentration inside the package (%), A is the area of the film (m^2) = length × width × 2, W = filling weight (kg), PO_2 is the required permeability for O_2 (ml O_2/(m^2 24 h atm)) at 7 °C and 90% RH, RO_2 is the respiration rate of the packaged vegetable, expressed as the O_2 consumption (ml O_2/(kg h)), R * O_2 is the pre-exponential factor for respiration (ml O_2/(kg h)), $E^R O_2$ is the activation energy for respiration (J/mol), R is the gas constant (8314 J/(mol K)), T is the environmental temperature (K), P * O_2 is the pre-exponential factor for O_2 permeability (ml O_2/(m^2 24 h atm)), $E^P O_2$ is the activation energy for permeability (J/mol), V_{max} is the maximal respiration rate (ml O_2/(kg h)) and K_M is the Michaelis–Menten constant (O_2 concentration at half of maximum respiration rate, %), V_1 is the pre-exponential factor of the maximal respiration rate (ml O_2/(kg h)) and V_2 is the temperature inverse (1/K).

The temperature dependence of RO_2, PO_2 and V_{max} is known and quantified (Eqs. (2), (3) and (5)). K_M is temperature-independent for the considered vegetables (Jacxsens et al., 2000a,b). Other coefficients were constant (R, $(O_2)_{out}$) or chosen (A, T, W). The unknown coefficient $(O_2)_{in}$ can be derived from this system at any temperature.

2.5. Microbiological analysis

The growth of relevant spoilage microorganisms was followed by classical enumeration methods in duplicate. After preparation of a dilution series, samples were pour-plated or spread-plated on specific media. Total aerobic psychrotrophic count was pour plated on plate count agar (PCA, Oxoid, CM325) and incubated at 22 °C for 5 days. MRS (de Man, Rogosa, Sharpe, Oxoid, CM361) + top layer was applied to count microaerophilic lactic acid bacteria after 3 days incubation at 30 °C. A spread plate with oxytetracycline glucose agar (OGA, Diagnostics Pasteur, Marnes-la-Coquette, France, 64894) with an additional supplement (oxytetracycline supplement, Oxoid SR073A) was used to allow enumeration of yeasts (after 3 days incubation) and moulds (after 5 days of incubation) at 30 °C. To evaluate the microbial load of the ready-to-use vegetables, critical limits are used as proposed by CNERNA-CNRS (1996) and Debevere (1996)

and as applied in previous work (Jacxsens et al., 1999a).

To enumerate *L. monocytogenes*, *Listeria* selective agar base (Oxford formulation, Oxoid, CM856 + *Listeria* selective supplement, Oxford formulation, Oxoid, SR140E) was applied and the spread plates were incubated at 37 °C for 48 h. *A. caviae* was spread-plated on modified bile salts irgasan brilliant green agar (mBIBG) (pH = 8.7) and incubated at 30 °C for 24 h (Neyts et al., 2000).

2.6. Evaluation of sensorial quality

The characteristic sensorial properties of each type of produce were followed during the simulated chill chain at different temperatures. The evaluation was performed by a trained sensory panel (8–10 people) by a descriptive test. The use of specially trained panels, which describe their reactions to a product, represents a means of obtaining product attribute information independent of any preference. In contrast, consumers usually tend to use quality and preference in confusion (Willocx, 1995). The organoleptical properties (taste, smell/flavour and crispness) were judged under IR light in a taste room. Additionally, visual characteristics (colour of the cut surfaces, dryness/transparency, general appearance) were scored under normal light. The scale is a numerical summary of a subjective evaluation that considers the severity of the symptom (Kader et al., 1973): rating from 1 (excellent) to 10 (extreme bad). Cut off score was fixed at 5. The fresh-cut vegetable was considered as unacceptable when a mean score above 5 was reached.

3. Results and discussion

3.1. Flux in headspace gas concentrations

Headspace gas composition (O_2 and CO_2) varied with changing storage temperature (Fig. 1). In general, the applied mathematical system was able to predict the evolution of the O_2 concentration in the tested packaging configurations. A difference between the predicted and actual O_2 concentration was found at the moment of packaging (time = 0 in Fig. 1) as initially a lower O_2 concentration was injected during packaging (moment of sampling 0). An O_2 concentration of 2.65% was expected after the moment of purchase by the consumer (20 °C), which did not correspond with the measured value ($3.40 \pm 0.39\%$ O_2) (moment of sampling 5). Apparently, the high temperature situation was too short to result in such a temperature increase (average temperature of the lettuce during the 2 h storage at 20 °C was 16.32 °C) and such an O_2 drop. The applied integrated system does not include a time-changing simulation

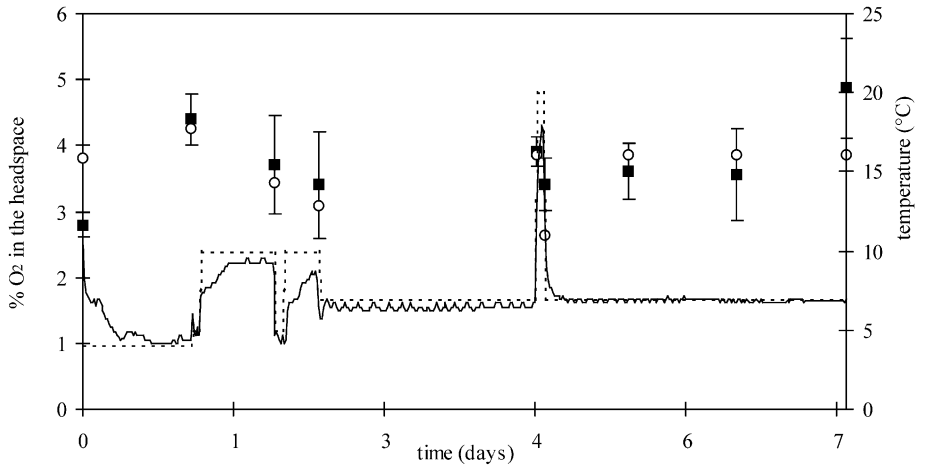

Fig. 1. Measured (■ ± standard deviation) and predicted headspace O_2 concentration (○) in EMA packages of mixed lettuce, followed through a simulated cold chain for ready-to-eat vegetables (——, product temperature; · · · · · ·, air temperature).

but simulates specific moments of time (Jacxsens et al., 2000a,b). At the end of the storage period (moment of sampling 8), the lettuce was found to respire less intense resulting in an underestimation of the headspace O_2 concentration. Respiration rate is decreasing due to tissue senescence (Kays, 1991). Throughout the simulated cold chain, the O_2 concentration remained between its acceptable limits ($>2\%$ to $<10\%$, Kader et al., 1989) although different periods of temperature abuse ($T>10$ °C) were included in the simulation. No anoxic conditions were created in the selected EMA packages, which is also found for other types of investigated vegetables in the temperature range of 2–10 °C (Jacxsens et al., 2000a,b). The CO_2 concentration, on the other hand, stayed stable between 3.5% and 3.9% CO_2. This low accumulation of CO_2 in the applied types of packaging films was also reported in other validation tests (Jacxsens et al., 1999a, 2000a,b). For the other two investigated ready-to-eat vegetables, a similar modification in headspace gas concentration was found although for bell peppers the predicted O_2 concentration was systematically higher possibly because of the O_2 consumption of the high microbial load.

3.2. Study of spoilage microorganisms

Growth rates of total psychrotrophic count (TPC), lactic acid bacteria (LAB) and yeasts were, in correspondence with other storage experiments with ready-to-eat vegetables, influenced by temperature and product type (Francis et al., 1999; Jacxsens et al., 1999a; Kaneko et al., 1999).

TPC of cucumber slices was increasing from an initial count of 2.42 ± 0.92 to 6.13 ± 0.03 $\log_{10}$ cfu/g after 7 days of storage in the simulated distribution chain. TPC of the cucumber slices was completely dominated by lactic acid bacteria (varying from 2.44 ± 0.86 to 6.19 ± 0.02 $\log_{10}$ cfu/g). The critical limits of these two groups of important spoilage microorganisms for vegetables of 10^8 cfu/g for TPC and 10^7 cfu/g for LAB were not exceeded during the storage in the simulated cold chain (CNERNA-CNRS, 1996; Debevere, 1996). The critical limit for yeasts of 10^5 cfu/g, on the other hand, was reached at the last day of the experiment when the EMA packages of cucumber slices were stored for 3 days in the refrigerator of the consumer (moment of sampling 8). This pronounced outgrowth of yeasts compared to LAB and TPC can be explained by the fact that the surface of the thin slices was drying out during storage.

The mixed and shredded bell peppers started with a rather high initial contamination in TPC (5.47 ± 0.19 $\log_{10}$ cfu/g) and again these were dominated by LAB (5.43 ± 0.10 $\log_{10}$ cfu/g). LAB and yeasts grew very fast on the mixed bell peppers. The limit of 10^7 cfu/g LAB was reached at the moment of sampling 2, but a higher number of LAB can be tolerated when sensorial properties are still acceptable (Kakiomenou et al., 1996). The mixed bell peppers were rejected based on their sensorial quality (texture loss) at the moment of sampling 4. At that moment, an amount of 10^8 cfu/g in LAB was counted, which can cause sensorial deterioration. Also, TPC and yeasts exceeded their critical limits at moment of sampling 4. The fast proliferation of the spoilage microorganisms can be explained by the fact that the mixture of green, red and yellow bell peppers was shredded in very small pieces (4×10 cm) providing water and nutrients to the microorganisms.

The evolution of the spoilage microorganisms on the mixed EMA lettuce is illustrated in Fig. 2. The initial contamination in TPC of 6.34 ± 0.18 $\log_{10}$ cfu/g is comparative with the values found by Kaneko et al. (1999) during an extensive research of the bacterial quality of retail ready-to-eat foods, where 77.8% of the tested raw vegetables cut for salad yielded TPC $>$ 5.0 $\log_{10}$ cfu/g. At the moment of purchase by the consumer (at moment of sampling 4), the critical limit of 10^5 cfu/g was exceeded for yeasts. The critical limit of TPC was, on the other hand, exceeded at day 6, after 48 h of storage in the consumers' refrigerator (moment of sampling 7). A 1.7 $\log_{10}$ cfu/g, increase in TPC was found between the moment of packaging (moment of sampling 0) and the 2 days of storage at the consumers home (moment of sampling 7). For LAB, no critical limit was reached. The selected time–temperature conditions represent a worst-case approach of the cold chain conditions and exaggerate the normal storage conditions of minimally processed vegetables. Willocx (1995) reported that more than 50% of the packages were sold during the first day of display in the supermarket. At that moment, an acceptable microbial quality was found for all three investigated fresh-cut vegetables. A sharp increase in spoilage microorganisms was detected after the moment of purchase and

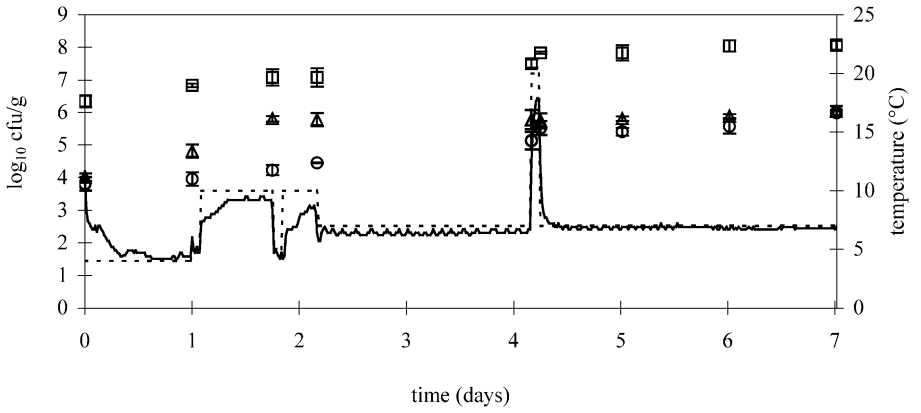

Fig. 2. Evolution of spoilage microorganisms on EMA-packaged mixed lettuce, followed through the simulated distribution chain (□ total psychrotrophic count ± 95% confidence interval; 333 ○ yeasts ± 95% confidence interval; △ lactic acid bacteria ± 95% confidence interval).

transportation to the domestic refrigerator (2 h, 20 °C) for all investigated fresh-cut vegetables (moment of sampling 5). The consumer is a weak link in the cold chain (Hurst and Schuler, 1992). Refrigerated foods are carried home unprotected and stored at too high temperature in the domestic refrigerators. By 6 out of 16 consumer refrigerators tested in Belgium in 1995, the minimally processed vegetables are stored at an average temperature above 7 °C (Willocx, 1995). In addition, the consumer is not aware of his contribution to the quality loss of the food products.

3.3. Safety of EMA-packaged ready-to-eat vegetables followed through the cold chain

The artificially inoculated psychrotrophic pathogens (cocktail of *L. monocytogenes* and *A. caviae*) were not able to multiply or even declined after 6 days of storage (*L. monocytogenes*) and after 2 days of storage (*A. caviae*) on the mixed bell peppers, packaged under EMA. The reason for this can be found in the high load of spoilage microorganisms dominated by LAB and the combination of refrigeration and the limiting pH of bell peppers (pH of the mixture = 5.07) for both *L. monocytogenes* ($pH_{minimum}$ for growth = 4.6–5.0) (ICMSF, 1996) and *A. caviae* ($pH_{minimum}$ for growth = 4.5–5.5) (ICMSF, 1996). For mixed lettuce, a small increase of the numbers of *A. caviae* was found while a significant increase in both pathogenic microorganisms (Table 3) was noticed in cucumber slices. Note that the growth rate and generation time are determined from a proliferation during changing time–temperature conditions.

The protective influence of a low natural pH of the ready-to-eat vegetables on the growth of both investigated psychrotrophic pathogens was obvious. The applied modified atmosphere in the packages (2–5% O_2 and 3–5% CO_2) did not affect in a direct way the growth/survival/declination of the pathogens (Berrang et al., 1989a,b; Bennik, 1997; Jacxsens et al., 1999a). For *Aeromonas* spp., no critical limit is proposed until now. A recent screening for the bacterial quality in

Table 3
Growth rate (log_{10} cfu/day) and generation time (h) of *L. monocytogenes* and *A. caviae* on EMA-packaged minimally processed vegetables subjected to a simulated distribution chain

Type of ready-to-eat vegetable	pH	*L. monocytogenes*		*A. caviae*	
		Growth rate (log_{10} cfu/7 days)	Generation time (h)	Growth rate (log_{10} cfu/7 days)	Generation time (h)
Cucumber slices	6.24	2.39	21.0	4.56	11.2
Mixed lettuce	5.76	*	*	0.58	84.0
Mixed bell peppers	5.07	*	*	*	*

* No significant growth ($P < 0.05$).

minimally processed lettuce showed the high incidence of this microorganism as 55% of the samples ($n = 120$) were positive for *A. hydrophila/caviae* (Szabo et al., 2000). *L. monocytogenes* was isolated from 2–5% of the samples surveyed by Szabo et al. (2000). Similar studies undertaken in France, England and Germany reported an isolation rate of 0–19% for *L. monocytogenes* (Nguyen-the and Carlin, 1994). It is very difficult to completely eliminate the presence of *L. monocytogenes* from a product like minimally processed vegetables, given its ubiquitous distribution. Consequently, there is a strong need to quantify the potential for its growth in packaged fresh-cut vegetables in order to accurately assess the risks associated with its presence in the product (Szabo et al., 2000). The absence in 0.01 g is postulated for *L. monocytogenes* at the end of the shelf-life of the ready-to-eat vegetable (CNERNA-CNRS, 1996; Debevere, 1996). Taking the observed growth rate of 2.39 $\log_{10}$ cfu/7 days of *L. monocytogenes* into consideration on cucumber slices, an absence in 2.45 g cucumber slices is necessary to satisfy the absence in 0.01 g after 7 days of storage in the simulated distribution chain.

3.4. Changes in sensorial shelf-life

The sensorial shelf-life was evaluated on both organoleptical and visual properties. For packaged products, such as minimally processed vegetables, appearance is the first and most important quality attribute evaluated by the consumer, and plays a major part in the decision of purchasing a food product (IFT, 1990). The aroma, flavour and mouth feel/texture properties will influence the evaluation of the consumer during consumption.

The EMA-packaged mixed lettuce was rejected on day 5 (after 1 day of storage in the consumers' refrigerator, moment of sampling 6) by 50% of the panel (mean score > 5) based on undesired colour modifications. Endive and lollo bionta in the mixed lettuce are very sensitive to enzymatic browning. Only complete removal of O_2 can avoid completely the phenolic oxidation catalysed by polyphenol oxidase. However, as such, anaerobic conditions are created resulting in other detrimental effects on organoleptical quality by accumulation of ethanol and acetaldehyde (Kader et al., 1989). After 1 day of storage in the consumers' refrigerator (moment of sampling 6), the properties taste, odour, and general freshness were judged with a mean score of 5.0 (just acceptable). On the second day of storage at the consumers' home, the lettuce was rejected by 75% of the panel. This implies that, for the investigated *t/T* chain, the fresh-cut vegetable should be consumed as soon as possible after purchase.

The panelists rejected the EMA-packaged shredded mixed bell peppers at 4 days of storage in the simulated distribution chain (100% would not consume the sample anymore) (moment of sampling 4). At that moment, the bell peppers are bought by the consumer in the supermarket. This rejection corresponds with exceeding the critical limit of 10^8 cfu/g in TPC/LAB. The main sensorial problem existed in their visual appearance/loss of texture due to an extensive water loss of the little cubes.

The evolution of the most significant sensorial properties of the EMA-packaged cucumber slices in function of the different steps in the cold chain is illustrated in Fig. 3.

In this radar-type of graph, the evolutions of the sensorial properties are illustrated as a function of the different sampling moments (indicated in Table 1). The different concentric lines are corresponding from inside to outside with the different sampling moments (0 to 6). The cut-off score of 5 is exceeded at days 3–4. At that moment, the microbial quality was still acceptable but apparently, the taste and the general

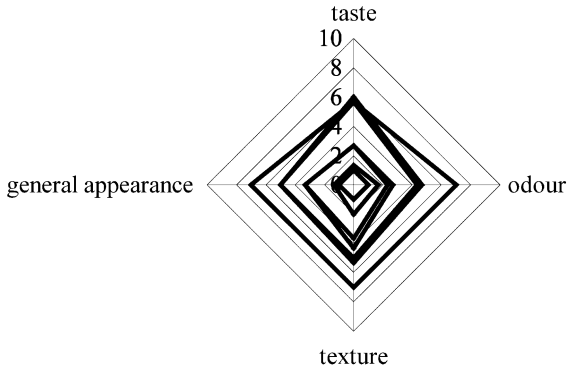

Fig. 3. Evolution of the main organoleptical properties (taste/odour/texture (mouth feel)) and general appearance (visual properties) of cucumber slices, packaged under EMA, and subjected to a distribution chain simulation.

Table 4
Microbial and sensorial shelf-life of fresh-cut vegetables, subjected to a "worst-case" cold chain simulation

Type of vegetable	Microbial shelf-life (days)	Sensorial shelf-life (days)	Safety conclusions (*L. monocytogenes*)
Mixed lettuce	4 (yeasts)	5 (colour)	– *
Mixed bell peppers	4 (yeasts, LAB, TPC)	4 (visual appearance/texture loss)	– *
Cucumber slices	7 (yeasts)	4 (taste/general appearance)	absence in 2.45 g at day 0 (day of production)

* No significant growth ($P < 0.05$).

appearance were not acceptable anymore for the panel (mean score > 5) because of the dryness of the thin slices.

4. Determination of the shelf-life of fresh-cut vegetables

Shelf-life should always be determinated based on both the microbial and the sensorial quality criteria. A maximum allowable microbial limit defines the end of the microbial shelf-life of a food product while the end of the sensorial shelf-life depends on the mean score of the individual properties and on the proportion of the consumer panel that will accept or reject the food sample for consumption. Table 4 summarises both shelf-lives for the three investigated types of vegetables.

5. Conclusions

Temperature abuse ($T > 10$ °C) can be found in a cold chain of minimally processed vegetables during transport and unloading at the supermarket, storage and display in cabinets and finally in the domestic refrigerators. Storage temperature is very important to guarantee microbial quality and safety of minimally processed vegetables. No anoxic conditions were found in this simulation of a "worst-case" cold chain by the application of high permeable packaging films. It was possible to predict the internal O_2 concentration with the integrated mathematical system as proposed by Jacxsens et al. (2000a,b).

The microbial shelf-life of minimally processed vegetables is limited mainly by the growth of yeasts. For mixed lettuce and mixed bell peppers, this was at the moment of purchase by the consumer (moment of sampling 4). Cucumber slices remained longer in a better condition and were acceptable until the last analysed day (day 7). When the microbial criteria were exceeded, also the sensorial properties were not acceptable anymore.

Less problems were found towards possible outgrowth of artificially inoculated pathogens. The combination of refrigeration and low pH did not allow *L. monocytogenes* to multiply on minimally processed bell peppers. Only a survival was detected on mixed lettuce. For the cucumber slices, on the other hand, safety precautions have to be taken in consideration. *A. caviae* was multiplying faster but was inhibited as well by the low pH of bell peppers.

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