GROWTH OF SPOILAGE MICROORGANISMS ON VEGETABLES IN A HIGH OXYGEN MODIFIED ATMOSPHERE SYSTEM IN RELATION WITH KEEPING QUALITY

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Abstract

Minimally processed vegetables are popular convenient products. Refrigeration and Modified Atmosphere (MA) packaging extend their shelf-life, but growth of spoilage and pathogenic microorganisms is often not inhibited. This study set out to determine whether a novel type of MA-packaging, referred to as high O_2-MA packaging, might allow a better control. Sliced minimally processed carrots were stored under high O_2-high CO_2 and changes of the total plate counts, pseudomonads, lactic acid bacteria were recorded after incubation at 8°C at high O_2 (50 or 90%), combined with CO_2 (10 to 30%). Exposure to the novel MAP did not affect growth of Enterobacteriaceae but caused changes in the subpopulation of lactic acid bacteria and pseudomonads. The effect of high oxygen MAP on *Leuc. mesenteroides,* a microorganism related to the preservation of sliced carrots, was tested on a surface model system. Total suppression of microbial growth was not obtained at the conditions studied. The impact of the gas compositions on microbial growth was assessed on the basis of general growth characteristics and quantified using mathematical modelling to derive specific growth parameters (lag phase duration, maximum growth rate and maximum yield). Improvement of several quality aspects (texture, water loss, colour) under high oxygen -MAP are discussed.

Introduction

The importance of modified atmosphere (MA) packaging for keeping quality and maintaining of the initial quality of perishable foods is now well established (Giese, 1997). For modified atmospheres of respiring products such as raw or minimally processed vegetables, diferent combinations of low O_2, (typically 2-3%) and moderately high CO_2 at equilibrium are employed. These conditions reduce physiological spoilage by limiting product respiration and maturation (Gorris and Peppelenbos, 1992) as well as by slowing down the proliferation of aerobic spoilage microorganisms (Kader et al., 1989).

MA packaging of respiring produce has come to substantial use in practice, but some potential problems with regard to product quality and safety remain to be solved. One of these is the difficulty to predict the gas composition during storage (Ahvenainen, 1996) due to the active respiration of the produce. Frequently, O_2 is completely depleted, resulting in a fermentation response of the product and the production of off odours. In addition, excessive levels of CO_2 (over 20%) cause specific physiological disorders. Recent studies in our laboratory (Bennik, 1997) have shown that spoilage flora is only partially suppressed at optimal for minimally processed vegetables, MA conditions.

Concerning product safety, psychrotrophic, facultatively aerobic pathogens (such as *Listeria monocytogenes*), may be enhanced in certain cases (Bennik *et al.*, 1996), especially because the MA conditions lower growth of spoilage microorganisms that would otherwise be competitors for the pathogens. Evidently, alternatives to the current, low oxygen MA packaging need to be investigated to better asure the safety of MA packaged respiring produce.

Recent experimental trials on fresh commodities have indicated that high O_2 (50%-90%) may be advantageous for product quality (Day, 1996). In the work presented here, the impact of the high oxygen-MAP (50-90%) in combination with high CO_2 (10-30%) on the growth of lactic acid bacteria, spoilage pseudomonads, and enterobacteriaceae on fresh, minimally processed carrots stored under controlled atmospheres was measured and quantified. Because surface contamination is most relevant for such products, we used a model surface system that exclusively allows surface growth, to show that combinations of high O_2 and C $_2$ inhibit or do not affect growth of several spoilage and pathogenic microorganisms (Amanatidou *et al.*, 1997). In the paper presented here, the surface model system is used to study the effect on pure cultures of *Leuconostoc mesenteroides*. The system was incubated at 8°C, a refrigeration temperature generally used for retail storage. In order to evaluate the data for the microbial growth the D-Model (Baranyi & Roberts, 1994) was used. Parameters related to the sensorial quality of the products (visual quality, weight loss, white discoulouration and texture) were also investigated.

Materials and methods

Minimal processing of carrots.

Carrots were purchased from a local retail shop and transported to the laboratory. They were disinfected by dipping into 10% H_2O_2 for 2 min and subsequently rinsed with sterile distilled water. The carrots were sliced with a domestic cutting machine. Subsequently 65 grams of sliced carrots were transfered in plastic boxes, placed in containers (20l vol.) and flashed continuously with a mixture of gases consisted of 1, 20, 50 or 90%, O_2 and 0 10 or 30% CO_2. A mixture of 20% O_2:80% N_2 was used as control.

Bacterial growth estimation. At certain time intervals three boxes were removed from each container. Total viable counts on PCA, lactic acid bacteria on MRS, yeast on OGGA, pseudomonads on CFC and enterobacteria on VRBGA medium were defined.

Inoculation of carrot disks A suspension of *Leuc. mesenteroides* was diluted in order to obtain a final population of 10^{-4} cfu.cm^{-2} of carrot surface. The suspension was sprayed onto the surface of the sliced carrots. The inoculated carrots were weighted and transfered in the containers as described before

Results

Effect on carrots

General growth characteristics

Pseudomonads, lactic acid bacteria and enterobacteriaceae made out the dominant flora of sliced carrots, were kept at different gas conditions after 9 days of storage at 8°C. The highest total viable counts were encountered for the samples kept under air after 5 days (8.3 log cfu.cm^{-2}). Fluorescent pseudomonas (mainly *Ps. fluorescens*), were present at very high levels already at the beginning of storage. Inhibition of *Pseudomonas spp.* was observed at 50% O2:30% CO_2 and at these conditions lactic acid bacteria (mainly *Leuconostoc* spp.) were predominant (Fig.1a). Significant was the reduction of lactic acid bacteria observed after 7 days under air (Fig 1b). Enterobacteria that were present at very low levels at the begining of storage increased more than 3-4 log cfu already after two days at all conditions tested. Reduction was only observed under high O_2-high CO_2 after 9 days. Maximum population densities of Enterobacteriaceae never exceeded the level of 6.5 log cfu cm^{-2}. Growth rates under control conditions (20% O_2:80% CO_2) were very high for all groups tested. Growth rates of *Pseudomonas* spp. were very low: 0.001 h^{-1} and 0.004 h^{-1} for 90% O_2:10% CO_2 and 50% O_2:30% CO_2 respectively. In the last case, an extended lag phase (4 days) was observed. Lactic acid bacteria could grow very well at all conditions tested and the optimum was at 90% O_2:10% CO_2.

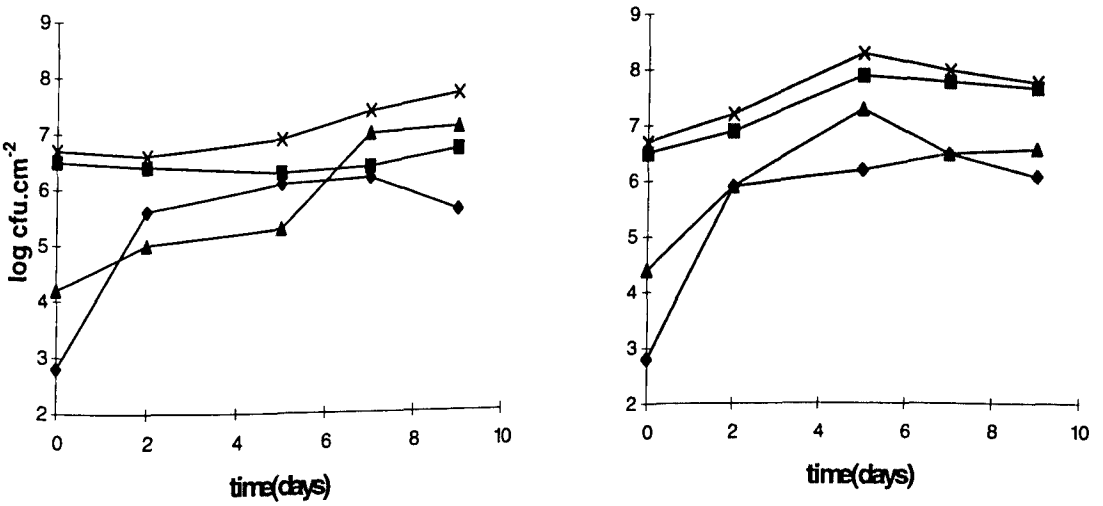

Fig 1 Changes in the microbial population of sliced carrots preserved under a) 30% CO_2:50% O_2 or b) 20% O_2:80 % N_2; υ Plate count on PCA ■ pseudomonads on CFC ▲ lactic acid bacteria on MRS and ◆ Enterobacteria on VRBGA

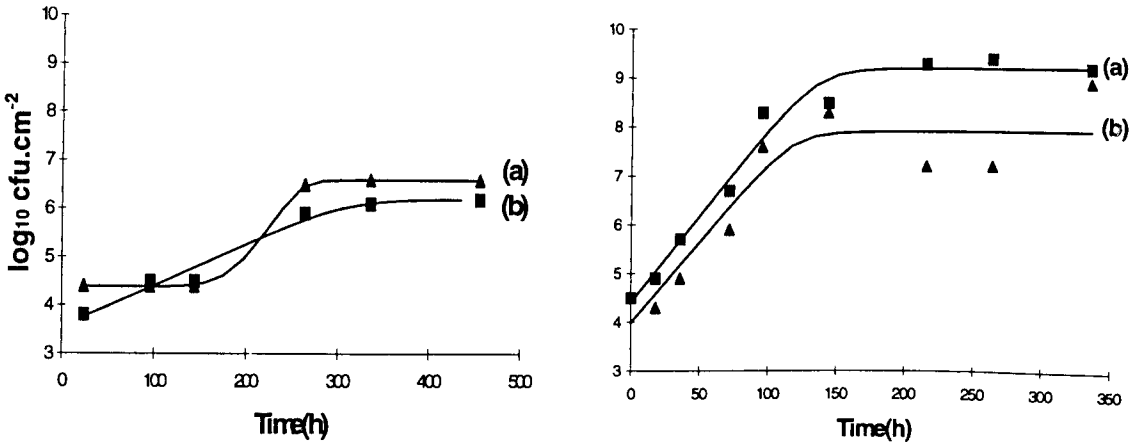

Fig 2 Growth of *Leuc. mesenteroides* subsp. *mesenteroides* (log cfu.cm^{-2}) inoculated on MRS plates and on sliced carrots after storage at 8°C ■ 90% O_2:10% CO_2 ▲ 20% O_2:90 % N_2 Data are fitted with the D-Model

Inoculation experiments and experiments on plates

In order to compare growth of *Leuconostoc mesenteroides* on carrots with growth on the surface model system both carrots and plates, were inoculated with a suspension of the bacterium up to a level of 10^{-4} log cfu.cm^{-2} and incubated at different conditions of O_2, CO_2 and N_2. Total counts for lactic acid bacteria on MRS from carrots and counts of the pure cultures of *Leuc. mesenteroides* inoculated on plates were compared. The D-model gave a good fit for the data from both carrots and plates (Fig 2).

General growth caracteristics

Lactic acid bacteria could grow very well on products at all conditions tested. In the case of 30% CO_2:70% N_2 lactic acid bacteria were clearly the dominant microorganisms but spoilage due to slime formation on the surface of the product was obvious after 7 days of preservation. Under 30% CO_2:50% O_2 lactic acid bacteria were predominant without slime formation even after 14 days of storage.

Maximum specific growth rate

Leuc. mesenteroides could grow rapidly on plates, especially under combinations of high O_2 high CO_2. The μ_{max} of lactic acid bacteria on carrots was 50-60% reduced comparing to the control, especially under 90% O_2:10% N_2, 80% O_2:20% CO_2 (table 1). On plates at concentrations higher than 60% a linear decrease of the μ_{max} was observed at increasing of *Leuc. mesenteroides* concentrations of CO_2 (data not shown).

Lag phase

Lag phase was apparent for lactic acid bacteria on inoculated carrots at high O_2-high CO_2 concentrations (100h) and on plates at 80% O_2:20% CO_2 (table 1).

Maximum population density

The MPD on plates was reduced to 7.3 and 7.9 log cfu.cm^{-2} for 80% O_2:20% CO_2 and 90% O_2:10% CO_2 respectively, compared to 9.2 cfu.cm^{-2} for the control.

Similar results were obtained for the growth on fresh products inoculated with *Leuc. mesenteroides.*

		Plates			Carrots		
O_2	**CO_2**	**μ_{max}**	**se**	**lag**	**μ_{max}**	**se**	**lag**
20	0	0.035	0.004	0	0.022	0.002	0
50	0	0.034	0.002	0	0.021	0.006	0
90	0	0.031	0.001	0	0.006	0.002	0
90	10	0.033	0.01	0	0.018	0.002	100
80	20	0.063	0.026	53	0.008	0.002	95
50	30	0.081	0.004	0	0.025	0.001	0
1	10	0.038	0.002	0	0.008	0.001	0
1	30	0.038	0.003	0	0.018	0.004	0

Table 1 Estimates of lag time (h) and maximum specific growth rates μmax (log cfu.ml^{-1}.h^{-1}) of *Leuc mesenteroides* subsp. *mesenteroides* on plates or minimally processed carrots for different concentrations of O_2 and CO_2 in the gas mixtures

Quality characteristics of carrots

White discouloration

Medium-white discoloration appeared already after seven days of storage. Due to this the product was unacceptable after 12 days of storage. Discoloration was more severe for products kept under ambient conditions (20% O_2).

Weight loss

The highest loss was observed for samples kept under 20% O_2:80% N_2 Under these conditions 10% of the weight was lost after 14 days of preservation. Samples under 10% CO_2:1% O_2 lost less than 5% of the initial weight. The losses under 90% O_2:10% CO_2 did not exceed the level of 5%.

Surface model system

The system used in this study has been described previously by Bennik *et al.* (1995) and modified by Amanatidou *et al.* (1997). In short, agar plates containing 9 ml of MRS agar were inoculated with a suspension of *Leuc mesenteroides* to an initial populaton of 10^3-10^4 CFU.cm^{-2} of plate surface and incubated in a series of 1-l jars. The jars were placed in a temperature controlled room at 8°C and connected in sequence to a gas flow-through system. The gas mixtures were prepared from pure gases using mass-flow controllers.

Microorganisms. Leuconostoc mesenteroides DSM 20343 was a type strain obtained from the Deutsche Sammlung fur Mikroorganismen und Zellkulturen (DSM). Stationary phase cells were harvested and washed twice in 0.1 M sodium phosphate buffer (pH 7.0) and then resuspended in the same buffer prior to use for inoculation experiments.

Quantification of viable counts. Plate samples were taken in duplicate at each sampling time by removing plates from two jars that were last in the line. The agar was removed aseptically from a plate and immediately homogenized with a stomacher. Subsequently, serial dilutions were made from the homogenates. The drop count method (Miles & Misra, 1938) was used to estimate the viable counts in cfu.ml^{-1}, which were converted to cfu.cm^{-2} assuming that all counts originated from microorganisms growing on the surface of the agar.

Quantification of microbial growth parameters

Viable count data were fitted to the mathematical model described Baranyi & Roberts (1994) and reparameterised the DMFit program (1996, IFR, Reading UK) which was kindly provided by Dr. Baranyi. The model was used to estimate the lag phase (h, hours), the maximum specific growth rate μ_{max} (h^{-1}) and the maximum population density y_{max} ($\log_{10}$ cfu.cm^{-2}).

Quality aspects of the product

In order to validate the effect of high oxygen MAP on the quality of the product the weight loss, white discouloration and the firmness of the products were examined. The firmness of the product was analyzed with the SMS TA XT2i Texture Analyzer time. 10 replicate samples were measured for each condition. Single sliced carrots were used. The tests were carried out with a 25 cm^2 compression anvil, cross head speed of 4 mm.sec^{-1} and a minimum clearance between anvil and platform of 2 mm. The results were analysed with a standard computer program.

Texture measurements

Carrots kept under 10% CO_2:1% O_2 were slightely softer compared to all the other conditions tested, but not significant differences were observed at the conditions tested.

Discussion

In this study, possible benefits of high O_2-MA packaging in reducing the growth potential of a number of spoilage microorganisms typically associated to minimally processed vegetables, as compared to the currently practiced low O_2-MA packaging were investigated.

Although maximum population density is not affected by high oxygen MAP changes in the subpopulation were apparent. In general, enterobacteria and lactic acid bacteria were not affected, growth of pseudomonads were significantly reduced. At 50%O_2:30%CO_2 growth of pseudomonads was totally inhibited.

The results about the effect of high CO_2 low O_2 atmospheres are in agreement with results from previous studies for chicory endive and mungbean sprouts (Bennik, 1997). Growth of pseudomonads which are obligatory aerobic microorganism is not affected at concentrations of oxygen as low as 1%. In these studies it was shown that the prevailing e of pseudomonas and enterobacteriaceae may exert a beneficial effect, due to their competition with occasionally occuring pathogens. In our study we showed that high oxygen atmosphere in combinations with moderate levels of carbon dioxide, play an important role in the formation of the final dominant flora. The importance of carbon dioxide for control of microbial growth is well established (Enfors and Molin, 1980, Eyles *et al.* 1993). Extention of lag phase seems to be a prominant effect of CO_2 (Farber, 1991). Is seems that this effect is stronger in the presence of high oxygen. On the other hand, it is well know that lactic acid bacteria can tolerate high concentrations of CO_2 and as shown in this study also of O_2.

Leuc. mesenteroides could grow rather well in the surface model system incubated at refrigeration temperature. On the bases of the current evaluation, we can conclude that there is hardly any correlation between the growth rates of this microorganism on the model system used and on sliced carrots. This can be explained by the complex nature of the substrate and by the interactions between the different subpopulations present on fresh produce. However, there was a good correlation between other growth characteristics like maximum population density and lag phase.

Growth of pathogens is of great concern for minimally processed vegetables. Amanatidou *et al.*, 1997, have shown that emerging pathogens like *Listeria monocytogenes* and *Salmonella* spp. remain unaffected or totally inhibited by high oxygen MAP on the surface model system. The effect of high oxygen MAP on the growth of food pathogens, on model carrots is currently under investigation.

The changes in the microbial population, the survival and growth of pathogens as well as other quality factors (like texture, colour,weight loss) will also be investigated in the future in a system of packed products. Based on the knowledge obtained so far, a model that can describe growth of food related microorganisms and especially pathogens but also the overall quality of the product will be developed for the determination of shelf life and safety of minimally processed carrots.

Acknowledgement

This work was supported by European Community through ERB40016T956040 and FAIR-CT96-1066. The authors gratefully acknowledge Dr J. Baranyi for fruitful discussions and Rob Slump for capable technical assistance.

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