Food Microbiology, 1996, **13**, 427–440

ORIGINAL ARTICLE

The influence of temperature and gas mixtures on the growth of the intrinsic micro-organisms on cut endive: predictive versus actual growth

K. Vankerschaver, F. Willocx, C. Smout, M. Hendrickx* and P. Tobback

Effects of temperature and carbon dioxide concentration on the growth of mesophilic aerobic count (MAC), psychrotrophic, Gram-negative bacteria, pseudomonads and lactic acid bacteria were investigated in a case-study of minimally processed endive. When plotting population density against time, a distinct lag time was not observed. In a temperature range from 4·3–16·1°C, both combined Arrhenius–Ratkowsky-restricted growth models were used to model growth of these groups of micro-organisms. Temperature dependence of the growth rate constant for pseudomonads and psychrotrophic, Gram-negative bacteria were similar, whereas the growth rate constant of the MAC was much more temperature dependent. A combined exponential (CO_2)–Arrhenius (temperature)-restricted growth model was selected to model the number of pseudomonads and an exponential (CO_2)–Ratkowsky (temperature)-restricted growth model was selected to model the growth of MAC on cut endive. The restricted growth model was differentiated with respect to time to obtain a dynamic restricted growth model. Temperature shift-up and shift-down experiments were performed. Use of the Arrhenius equation for the temperature dependence of the growth rate constant and parameter estimates obtained under constant CO_2 and temperature conditions only partially allowed us to predict the microbial evolution of minimally processed endive under dynamic conditions. Especially under temperature shift-down conditions, a systematic underestimation of growth was observed.

Received:
27 January 1996

Introduction

The food industry is aggressively seeking preservation technologies that deliver convenience products which look fresh and palatable. One of these 'new' technologies consists of refrigeration in combination with modified atmosphere packing (Map) of minimally processed vegetables.

The spoilage of chilled (minimally processed) foods is a complex phenomenon involving physical chemical, biochemical and biological changes. The principal spoilage mechanisms affecting minimally processed vegetables are microbial growth, oxidation (enzymatic browning) and moisture loss (Day 1992, O'Beirne 1990). To a large extent, the

Katholieke Universiteit Leuven, Department of Food and Microbial Technology, Laboratory of Food Technology, Kardinaal Mercierlaan 92, B3001 Heverlee, Belgium

*Corresponding author.

0740-0020/96/060427+14 $25.00/0

safety of minimally processed products depends on post-processing storage conditions which are seldom ideal at the industrial or home level (Evans et al. 1991; Willocx et al. 1994). The integrated effect of time and temperature allows organoleptic and nutritional quality to deteriorate, as well as the proliferation of pathogenic and spoilage micro-organisms.

The predominant microflora of fresh leafy vegetables are aerobic, psychrotrophic, Gram-negative bacilli, with counts of *Pseudomonas* and *Erwinia* spp. of approximately 10^5 cfu g^{-1} (Carlin et al. 1990, Denis and Picoche 1986, Picoche and Denis 1988). Variations of the storage conditions will alter not only the growth rate of micro-organisms, but also the type of spoilage micro-organisms that will predominate (Barriga et al. 1991, Brackett 1987, Sinell 1980). A shift in storage temperature and gas concentration in the package of minimally processed vegetables will shift the microflora towards lactic acid bacteria (Manvell and Ackland 1986).

In this study, the predictive performance of a combined temperature-growth model is evaluated for the total mesophilic aerobic count (MAC), the number of psychrotrophic, Gram-negative bacteria and the number of pseudomonads on minimally processed endive. The effect of the different raw materials, gas composition and temperature on the growth characteristics was evaluated. After validation, a suitable temperature dependence relation was used to predict the growth of different groups of bacteria under dynamic temperature conditions in order to evaluate the time–temperature tolerance hypothesis (McMeekin et al. 1993) for microbial growth on minimally processed endives.

Materials and Methods

Minimal processing of endives

The endives used in this study were processed in a standardized way. The following processing steps are similar to industry practices of minimally processed vegetables. On the first day, a tray of six chilled endives (with an average weight of ±900 g) was bought at a local vegetable auction and transported back to the laboratory within 35 min. Variety and experimental conditions of the endives are given in Table 1. The endives used in the first experiment (A) were bought in a local supermarket, and as a consequence, history and origin are not known. The endives that were bought at the vegetable auction, were harvested 2 days prior to purchase and were kept refrigerated (±4°C).

Upon arrival in the laboratory, the outer wrapper leaves were discarded and the endives were sliced with a sharp stainless steel knife into strips of about 1 cm. The pieces were rinsed twice with cold water (tap water, ±12°C), thoroughly mixed and centrifuged (manually operated) to remove excess water. In this study, the starting point of the microbial shelf life of the minimally processed endives was defined from the end of processing. After processing, 50 g of the endives were individually packed in polypropylene net bags (sterilized with alcohol). At least 15 packages were placed in a sealed container (20 l) fused with the appropriate gas mixture. The gas was passed through a water flask before entering, to avoid dehydration of the endive. In order to avoid gas composition changes due to respiration, the gas flow was adapted to the remaining packages in the container. The initial flow was set at 400 ml min^{-1} and decreased to 50 ml min^{-1} towards the end of the experiment (the flow was calculated to compensate for the normal respirational activity of the crops). A temperature data recorder (external sensor, sterilized) measured the temperature within the containers. The entrance hole of the sensor was also used as the venting hole of the container. The containers were stored at the desired temperature and gas composition for 11 days. Six independent experiments (indicated further as A, B, C, D, E and F) were carried out. The experimental conditions are listed in Table 1.

Microbial sampling plan

From the fresh minimally processed endive, the initial microbial load (time=0) was

Table 1. Variety and experimental conditions of the minimally processed endives; temperature (θ±s.d.) (°C) and gas mixtures

Exp.	Variety	Gas composition	Temperature (°C)	Sampling scheme (hours)
A	Unknown	Air	5·9 (±0·3) 10·7 (±0·3) 15·3 (±0·2)	0·24,48,72,96,168,192,216,240,264
B	Nuance	Air	4·9 (±0·3) 9·9 (±0·3) 16·1 (±0·3)	0,24,48,72,96,168,192,216,240,264
C	Nuance	Air	4·4 (±0·4) 10·1 (±0·3) 15·7 (±0·3)	0,24,48,72,96,120,144,168,192,216,240,264
D	Nuance	Air 11 (v/v) CO_2 19 (v/v) CO_2	8·1 (±0·3) 8·1 (±0·3) 8·1 (±0·3)	0,24,48,72,96,168,192,216,240,264
E	Nuance	Air	4·3 (±0·3) 15·8 (±0·3) 4(24h)–16(72h)–4(72h)–4(72h) 4(24h)–4(72h)–16(72h)–4(72h)	0,24,48,72,96,120,144,168,192,216,240,264
F	Nuance	Air	10·2 (±0·3)	0,24,48,72,96,168,192,216,240,264

enumerated in each experiment. Only one sample (50 g) was analysed in the first experiment (A), two samples in the second (B) and third (C) experiment and three samples in the fourth (D) and fifth (E) experiment. Each sample was homogenized in 450 ml sterile water in a Warning Commercial Blender (VEL, Belgium) for 50 s. Serial dilutions were made in a sterile Ringer solution (9 g NaCl l^{-1}) and the appropriate dilutions were plated in triplicate and enumerated using the procedures of Mossel and Jacobs-Reitsma (1990) as follows:

Mesophilic aerobic count (MAC). Pour plate (1 ml sample), enumerated on plate count agar (PCA Difco); pH: 7·0±0·2; incubated for 72 h at 30±1°C.

Psychrotrophic, Gram-negative bacteria. Spread plate (0·1 ml sample) enumerated on PCA (Difco) with 1 ppm crystal violet (UCB, Belgium), incubated for 72 h at 22±1°C.

Pseudomonadaceae (pseudomonads). Spread plate (0·1 ml sample), enumerated on selected *Pseudomonas* agar base (PA, Oxoid) supplemented with CFC (5 mg cetrimide; 5 mg fucidin; and 25 mg cephaloridine, per 500 ml sterile PA, Oxoid) and glycerol; pH: 7·1± 0·2; incubated for 48 h at 30±1°C.

Lactic acid bacterials (LAB). Enumerated on De Man, Rogosa and Sharp agar (MRS, Oxoid); pH: 6·2±0·2. After inoculation of the agar (1 ml sample), an overlay layer of MRS was added. The plates were incubated aerobically for 72 h at 37±1°C.

Pour plates giving 30–300 well-separated colonies were selected for enumeration, whereas for the enumeration of spread plates, petriplates in a suitable range from 15–150 cfu were used. During the storage experiment, the prepared packages of endive were removed from the container following a predetermined sampling scheme (see Table 1), and the same micro-organisms were tested as for the initial microbial load. The time of removal of the prepared package minimally processed endive from the container was identified as the time of sampling. The pH of the homogenized diluted endive sample (50 g in 450 g H_2O) was measured daily (WTW pH 535 multical, VEL, Belgium).

Data analysis

The temperature dependence regression coefficients were estimated with the one-step or global regression approach (Haralampu et al. 1985) using the procedure NLIN of the SAS software package (SAS 1982). The data set was considered as a whole and the regression coefficients were estimated through the combination of the temperature dependence relations for lag time and growth rate after substitution into the growth curves.

Equations/models used for predictive models in this study were:

(1) Model for restricted growth under isothermal conditions

$$Y = Y_m + i.\exp(-k_\theta.t) \qquad (1)$$

(2) Model for restricted growth under dynamic conditions

$$\frac{dY}{dt} = k_\theta . (Y_m - Y) = -k_\theta . [i.\exp(-k_\theta.t)] \qquad (2)$$

(3) The Arrhenius equation

$$k_\theta = k_{\theta ref}.\exp[(E_a / R).(1/\theta_{ref} - 1/\theta)] \qquad (3)$$

(4) The Ratkowsky equation (Ratkowsky et al. 1982)

$$k_\theta = [b_{rat} . (\theta - \theta_{min})]^2 \qquad (4)$$

where Y is the bacterial population density at time t [log(cfu/g)]; Y_m is the log(N_{max}), maximum population density or saturation level [log(cfu g^{-1})]; i is equal to the [log(N_0)–Y_m], negative increase in microbial count; [log(cfu g^{-1})]; N_0 is the initial population density at time t=0 (cfu g^{-1}); k_θ is the growth rate constant (h^{-1}) at a temperature θ [°C]; t is the time (h); N is the bacterial population density at time t (cfu g^{-1}); θ is the temperature [°C or K]; θ_{min} is the theoretical minimum temperature for growth [°C or K]; θ_{ref} is the

reference temperature [°C or K]; $k_{\theta ref}$ is the maximum growth rate at reference temperature h^{-1}; E_a is the activation energy or temperature characteristic (J mol^{-1}); R is the universal gas constant (8·314 J mol^{-1} K^{-1}); b_{rat} is the Ratkowsky regression parameter ($K^{-1}.h^{-0·5}$).

Results and Discussion

Initial microbial load of minimally processed endive

The initial microbial load of the minimally processed endive was the starting value (input variable) of the predictive model. Furthermore, in order to be of value, it was necessary to estimate the contribution of the number of pseudomonads to the total population. Therefore, the number of pseudomonads was compared with the MAC and the number of psychrotrophic, Gram-negative bacteria. The initial microbial load of the fresh washed and cut endive is given in Table 2. As can be seen from this table, the number of pseudomonads constituted a major part of the initial microflora of minimally processed endives, independent of the experiment. Moreover, the number of aerobic, mesophilic bacteria was comparable with the number of psychrotrophic, Gram-negative bacteria. In France, the acceptable microbial limit for MAC (m) equals 5·7 log(cfu g^{-1}) with an upper level (M) of 6·7 log(cfu g^{-1}) at the moment of production (Anon. 1988). To account for the variation in microbial enumeration techniques, by law, a deviation of ±0·5 log-unit from the lower limit is allowed. At the moment of consumption, the upper acceptable level, which should not be exceeded for minimally processed vegetables, equals 5·7 log(cfu g^{-1}). Deviations of the upper level, however are not allowed. Based on this three-class attributes sampling plan, the minimally processed endive used in this study would be of marginal quality (Jarvis 1989).

The initial number of lactic acid bacteria did not differ much from experiment to experiment, and varied in a range from 2·0–2·8 log(cfu g^{-1}). These rather low counts are in accordance with the levels found in lettuce (Denis and Picoche 1986).

Ideally, samples drawn from a lot should be representative of the whole lot. Because of the variation in the distribution of organisms in the food and the imprecision associated with the enumeration technique, representative sampling for solid foods is not an easy job. In order for the count to be of value for the model predictions, it is necessary to have an indication of the accuracy of the bacterial count. In the fourth (D) and fifth (E) experiment, three replicate samples were analysed to estimate the variance of the reported mean population density. In Table 3, the variability of the different microbial counts are given. Note also that the mean microbial count was the average of three plate counts.

The reported mean values are repeatable within 10% of the values obtained from another sample. Furthermore, the reported standard deviation on the microbial count is in accordance to the overall 95% confidence limit of ±0·5 log-units of the mean value obtained from replicate tests on a single sample (Jarvis 1989). As can be seen from

Table 2. Initial microbial load [mean values, log(cfu g^{-1})] of minimally processed endive, with n, the number of replicate samples

Micro-organisms replicate samples	Experiment				
	A 1	B 2	C 3	D 4	E 5
MAC	N.C.[a]	5·72	6·20	5·81	5·83
Psychrotrophic Gram-negative	6·00	5·53	5·65	5·86	5·53
Pseudomonads	5·94	5·36	5·34	5·58	5·08
LAB	2·00	2·43	2·63	2·40	2·76

[a]N.C.=Not Counted.
MAC, mesophilic aerobic count; LAB, lactic acid bacteria.

Table 3, the inter-sample variation (expressed as standard deviation) leads to even wider confidence limits. As a consequence, when comparing the observed microbial counts with the predicted levels, a broad confidence range must be taken into account to compensate for the variability of the analytical method and the variability due to the distribution of micro-organisms in the food sample.

Model for restricted growth

When taking a closer look at the microbial counts of the minimally processed endives, the change in microbial count was not a smooth sigmoidal curve. Moreover a distinct lag phase was not observed. Similar observations were made by Hudson and Mott (1993), who found that *Aeromonas hydrophila*, inoculated on samples of cooked beef, were not consistent with the Gompertz equation and did not show any lag time. Also Langton et al. (1993) found that *Salmonella* grown on chicken samples typically did not exhibit a lag phase.

By lack of a clear lag phase in the microbial growth curves of the different groups of micro-organisms on minimally processed endives, the restricted growth model (Eqn 1) appears useful. The model must be modified by using a logarithmic population density (log(cfu g^{-1})) instead of the absolute value. The restricted growth model describes an exponential saturation to a predefined maximum population density.

The effect of temperature and raw materials on the kinetic growth parameters

In order to verify the influence of variation of the raw materials on the kinetic growth parameters, the temperature dependence relations were evaluated between experiments.

Table 4 summarizes the one-step regression analysis for the number of pseudomonads using the Arrhenius (Eqn 3) and Ratkowsky (Eqn 4) equations in combination with the model for restricted growth. The activation energy for the pseudomonads population of experiments B and C was somewhat lower when compared with the activation energy estimated for the other two experiments (A and E). Conversely, the estimated growth rate constant at reference temperature (283·15 K or 10°C) of the experiments B and C is much higher. The same trend was observed in the parameter estimates of the Ratkowsky relation. The parameter values for experiment C deviates somewhat from the other parameter estimates. Although more data points were used in the regression analysis for experiment C, the high mean residual sum of squares (MRSS) indicates a poor goodness-of-fit.

In Table 4, the result of a global regression analysis using the entire data set is also given. If the MRSS is used as sole selection criterion for the goodness-of-fit, the combined Arrhenius-restricted growth model is favoured for the growth of pseudomonads on minimally processed endive. An average acti-

Table 3. Within and between sample variability (s.d.) of the number of pseudomonads, psychrotrophic, Gram-negative bacteria and mesophilic aerobic count (MAC) [log (cfu ml^{-1})]

Exp.	Sample	Pseudomonads mean±s.d.	Psychrotrophic Gram-negative mean±s.d.	MAC mean±s.d.
D	a	5·42±0·046	5·75±0·070	5·56±0·089
	b	5·76±0·080	5·96±0·018	5·92±0·029
	c	5·44±0·047	5·84±0·048	5·85±0·099
	total	5·54±0·17	5·85±0·1 0	5·77±0·18
E	a	5·26±0·028	5·66±0·038	5·83±0·032
	b	5·05±0·022	5·59±0·100	5·93±0·112
	c	4·80±0·039	5·19±0·056	5·80±0·185
	total	5·04±0·20	5·48±0·13	5·85±0·13

The mean value of the individual sample is calculated from three petri plates.

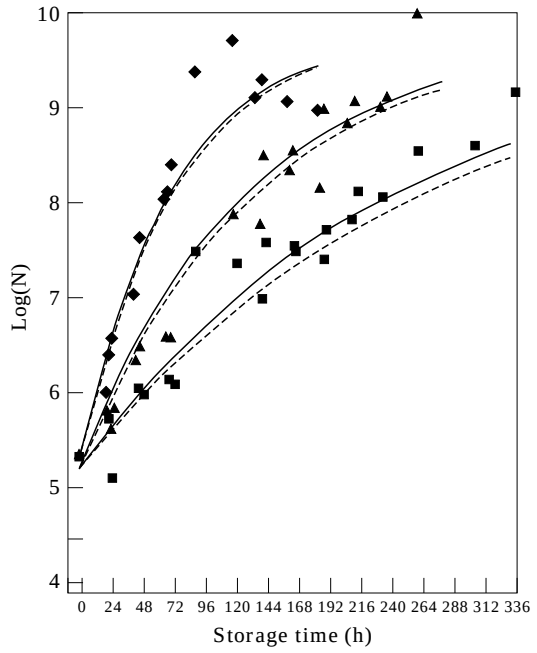

Figure 1. Number of pseudomonads (log(cfu g^{-1})) of experiment C, at 4·4, 10·1 and 15·7°C under air, with the predicted growth curves using the regression parameters of experiment C (–) and the average parameters from the entire data set (- - -) (Table 4). (■) 4·4°C, (▲) 10·1°C, (◆) 15·7°C.

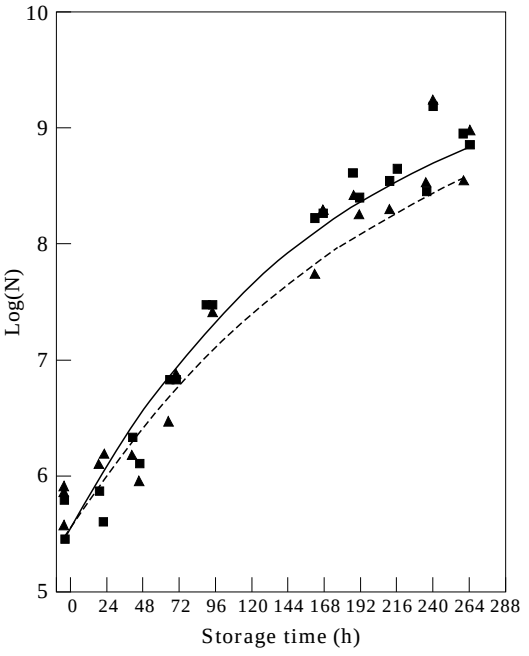

Figure 2. Validation step. Number of pseudomonads and mesophilic aerobic count (log(cfu g^{-1})) of experiment D (at 8·4°C, under air), with the predicted growth curves using the regression coefficients of the fitting sample. (■) Pseudomonads, (▲) mesophilic aerobic count.

vation energy of 76·7 kJ mol^{-1} and a growth rate constant of 0·00704 (h^{-1}) at 283 K was estimated (Table 4). If the selected growth model is independent of the raw materials used, the average parameter coefficients can be used to predict the growth of pseudomonads on the subset data. In an attempt to evaluate this hypothesis, the growth of the number of pseudomonads of a specific experiment was estimated using the regression coefficients of the entire data set (Table 4) and compared with the predictions using the regression coefficients of that specific experiment. Fig. 1 shows the experimental and predicted growth curves of the number of pseudomonads of experiment C. As can be seen at 15·7°C, both regression coefficients resulted in identical growth curves, whereas at 10·1°C and 4·4°C the growth was underestimated compared with the model estimates from the entire data set.

However, the difference between both model estimates was less than the measurement error; a systematic underestimation of the growth cannot be denied. It should be noted that the minimally processed endive taken in the verification step had regression coefficients that deviated most from the average of the entire data set.

Model validation

One can expect that if the model is not capable of accurately predicting the microbial evolution of a data set that was used with the fitting analysis, the prediction performance for an entirely new data set will be even worse. In order to validate the selected model (combined Arrhenius-restricted growth model) and to update the parameter estimates, additional validation experiments were undertaken. In Fig. 2, the experimental and predicted growth curve of the number of pseudomonads and MAC at 8·4°C under air of experiment D is given. The parameter estimates of the selected Arrhenius-restricted growth model are taken from Table 4. In Fig. 3, the number of pseudomonads and the

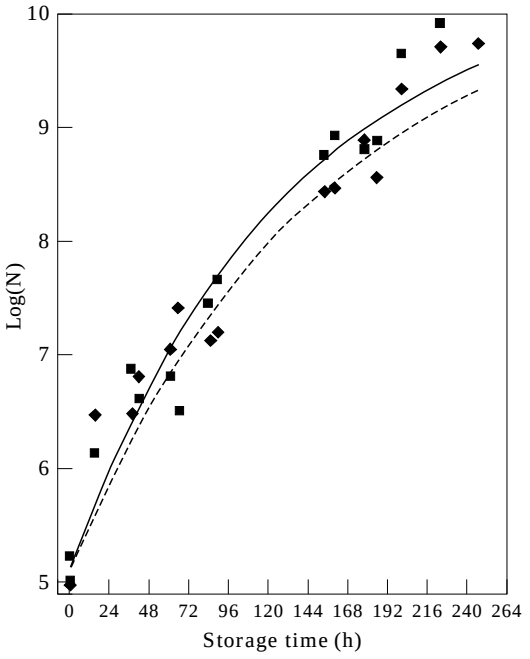

Figure 3. Validation step. Number of pseudomonads and psychrotrophic, Gram-negative bacteria (log(cfu g^{-1})) of experiment F (at 10·2°C, under air), with the predicted growth curves using the regression coefficients of the fitting sample. (■) Pseudomonads, (▲) psychrotrophic, Gram-negative bacteria.

psychrotrophic, Gram-negative bacteria at 10·2°C for the cut endive (variant Nuance) of an independent experiment (experiment F) not involved in the parameter estimation procedure is shown together with the predicted population density for both groups of micro-organisms. The regression coefficients for the MAC and psychrotrophic, Gram-negative population were also taken from a global analysis from the entire data set (not shown). As can be seen from Figs 2 and 3, the predictions of the number of pseudomonads, MAC and psychrotrophic, Gram-negative bacteria on the minimally processed endive are acceptable in both experiments.

These validation experiments confirm the hypothesis that the growth of groups of micro-organisms on minimally processed endives can be assumed independent of the raw materials used. Naturally, the raw materials will influence the initial flora, the availability of nutrients and the presence of competitive micro-organisms, but these factors are—or can be—hidden by the inaccuracy of the analytical counting method. Quality and quantity of the data are of paramount importance for both fitting and validation of the combined temperature growth model. For instance, in the experiment C, the population density counted in the stationary phase at 15·7°C varied between 9·75 log(cfu g^{-1}) at 124·32 h and 8·12 log(cfu g^{-1}) at 141·48 h (not shown). If the enumeration method in the fitting step is inaccurate, one can expect the models to be even more inaccurate. Furthermore, the measurement of the temperature of the minimally processed endive was difficult. Because almost 1 kg product was incubated initially, temperature was influenced by the respiration activity of the produce, which in turn was influenced by the incubation temperature. The fluctuations in temperature during storage is reflected by the high standard deviations of the mean value (Table 1). However, a precise measurement of the temperature is a prerequisite of the physical mathematical evaluation method of the preservation process. In order to evaluate the impact of the process on the quality attributes of the food product, the controlling physical parameters (e.g. temperature) must be determined accurately.

In Table 5, the updated parameter estimates of the combined Arrhenius- and Ratkowsky-restricted growth models are given for the number of pseudomonads, the psychrotrophic, Gram-negative bacteria and the MAC using both the fitting and validation sample data set. Based on the MRSS, it can be concluded that after updating, both combined Arrhenius- and Ratkowsky-restricted growth models can be used to model the growth of the groups of micro-organisms on minimally processed endive in a temperature range from 4·3–16·1°C. The temperature dependence of the growth rate constant for the pseudomonads and psychrotrophic, Gram-negative bacteria are similar, whereas the growth rate constant of the MAC is much more temperature dependent.

Modelling the effect of gas composition

Next to temperature, modified atmosphere is another growth controlling factor of

Table 4. Parameter estimates of the temperature dependence of the growth rate constant for pseudomonads (under air)

Exp.	E_a (kJ mol^{-1})	95% Cl	k_{283} (h^{-1})	95% Cl	MRSS	# points	b_{rat} (°C^{-1}.h$^{-0.5}$)	95% C.I.	θ_{min} (°C)	95% C.I.	MRSS	# points
A	84·03	69·73–98·32	0·00466	0·00427–0·00505	0·0859	42	0·00449	0·00373–0·00525	–5·63	–8·08– –3·19	0·0800	42
B	74·97	65·14–84·80	0·00796	0·00744–0·00847	0·0851	52	0·00504	0·00435–0·00574	–8·14	–10·41– –5·87	0·0890	52
C	72·71	61·68–83·75	0·00744	0·00689–0·00800	0·135	58	0·00451	0·00376–0·00527	–9·55	–12·45– –6·65	0·145	58
E	85·96	75·70–96·22	0·00703	0·00641–0·00765	0·123	41	0·00551	0·00475–0·00626	–6·16	–7·93– –4·39	0·123	41
Total	76·70	70·14–83·26	0·00704	0·00672–0·00736	0·173	193	0·00475	0·00431–0·00519	–8·19	–9·69– –6·69	0·178	193

Global regression analysis using the Arrhenius (Ea, k_{283}) and Ratkowsky ($brat$, θmin) relation in combination with the model for restricted growth.

Table 5. Parameter estimates of Arrhenius (E_a, k_{283}) and Ratkowsky (b_{rat}, θ_{min}) relation for the pseudomonads, psychrotrophic, Gram-negative bacteria and the mesophilic aerobic count (MAC) using the entire data set

Group	E_a [kJ/mol]	95% Cl	k_{283} [h^{-1}]	95% Cl	MRSS	# points	b_{rat} [°C^{-1}.h$^{-0.5}$]	95% C.I.	θ_{min} [°C]	95% C.I.	MRSS	# points
Pseudomonads Psychrotrophic	77·20	70·73–83·66	0·00720	0·00690–0·00750	0·169	230	0·00484	0·00441–0·00527	–7·98	–9·40– –6·55	0·171	230
Gram-negative	67·56	53·92–81·20	0·00651	0·00600–0·00701	0·258	114	0·00417	0·00333–0·00501	–9·65	–13·42– –5·87	0·255	114
MAC	102·39	90·69–114·08	0·00630	0·00577–000676	0·185	106	0·00609	0·00536–0·00682	–3·70	–5·05– –2·36	0·179	106

minimally processed vegetables. In this study, the number of pseudomonads and MAC of minimally processed endive stored under isothermal conditions (8·1°C) under 0·03% (air), 11 and 19% CO_2 were evaluated (experiment D). The global regression analysis, relating the growth rate of the modified model for restricted growth to the CO_2 concentration with a linear or exponential dependence relation is given in Table 6. It must be remembered that the sum of CO_2 and O_2 concentrations in the gas mixture equals 21% by volume. The initial microbial load is taken from Table 2 and the maximum population density is set at 9·8 log(cfu g^{-1}). For both groups of micro-organisms, the exponential dependence relation of the growth rate constant with the CO_2 concentration in the head space is selected as the best fitting model. For both models, the MAC is more retarded by an increased CO_2 concentration than the number of pseudomonads. In Fig. 4, the observed and fitted growth curves of the number of pseudomonads indicate that the predicted shelf life of minimally processed endive is almost doubled from 127 h stored under air to 230 h stored under 18·4% by volume CO_2 (and 2% O_2), when taking 7·7 log(cfu g^{-1}) as the acceptable limit for consumption. Further research to evaluate a possible synergistic effect of the gas composition on the natural microflora of minimally processed endive is necessary. For simulation studies, the CO_2 dependence relations (linear or exponential) were fit into a global CO_2-temperature–growth model. Based on the MRSS of Table 5 and 6, a combined exponential–Arrhenius-restricted growth model is selected for the number of pseudomonads and an exponential–Ratkowsky-restricted growth model for the MAC.

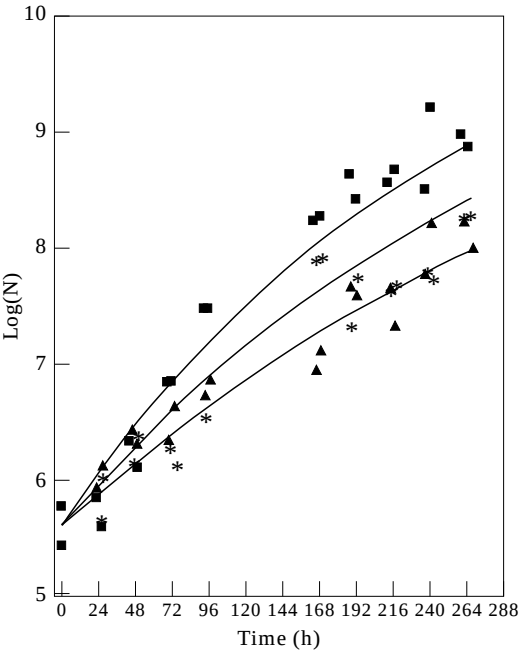

Figure 4. The observed and predicted number of pseudomonads (log(cfu g^{-1})) on minimally processed endive (experiment D) at 8·1°C under 0·03 (air), 10·6 and 18·4 vol% CO_2. (■) air, (▲) 10·6% CO_2, (*) 18·4% CO_2.

Modelling growth of the number of pseudomonads under dynamic temperature conditions

The modified model for restricted growth (Eqn 1) is the explicit solution of a more general dynamic differential equation in the special case of a constant temperature. By analogy of the derivation of the dynamic model from the original Gompertz equation, the model for restricted growth (Eqn 1) is differentiated with respect to time, as described in Eqn 2.

This dynamic model for restricted growth

Table 6. Global regression analysis for the CO_2 dependence relation (% (v/v)) of the growth rate constant (h^{-1}) in combination with the model for restricted growth for the number of pseudomonads and mesophilic aerobic count (MAC) at 8·1°C (experiment 13/09)

Micro-organisms	Model	Growth rate constant	MRSS
Pseudomonads	Linear	k_θ=0·00527–0·000120*[CO_2]	0·0837
	Exponential	ln(k_θ)=–5·210–0·0319*[CO_2]	0·0768
MAC	Linear	k_θ=0·00470–0·000156*[CO_2]	0·123
	Exponential	ln(k_θ)=–5·331–0·0347*[CO_2]	0·118

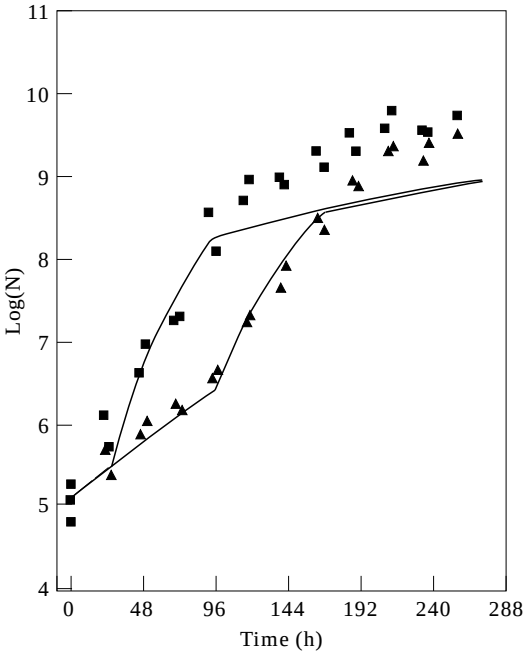

Figure 5. The observed and predicted number of pseudomonads (log(cfu g^{-1})) using the dynamic model for restricted growth in combination with the Arrhenius relation (Table 4) for two time–temperature profiles (experiment E). (■) 4-16-4-4°C, (▲) 4-4-16-4°C.

has the similar advantages as those reported for the dynamic model of Van Impe et al. (1992). When the time–temperature profile is divided into short, constant temperature intervals ($\triangle t_i$), and the optimal temperature dependence relation of the growth rate constant of the selected microflora is included, the population density of the minimally processed endive can be calculated by forward numerical integration.

Fig. 5 shows the number of pseudomonads of the dynamic part of experiment E, and the predicted growth curves using the dynamic model for restricted growth (Eqn 2) in combination with the Arrhenius equation for the temperature dependence of the growth rate constant. The data obtained at 4 and 16°C (isothermal conditions) were used to determine the temperature dependence constants (Table 4). After the first temperature shift-up (dynamic conditions), it was observed that the number of pseudomonads react immediately to the new incubation temperature, and the prediction was in close agreement with the observed microbial load. Because almost 1 kg produce was stored in the container, it took almost 4 h before the temperature inside the container reached the new incubation temperature. This temperature increase could hide a possible history effect, but nevertheless, the number of pseudomonads is nicely predicted after the temperature shift-up step. It has been shown that the culture status affects the duration of the lag phase, but does not alter the doubling time or maximum population density (Duncan and Nickerson 1962, George and Lund 1992, Jason 1983, Mackey and Kerridge 1988, Pamment et al. 1978).

After the temperature shift-down from 16 to 4°C, the number of pseudomonads was completely underestimated for both scenario's. The difference between the observed and predicted values cannot be related to the analytical measurement error; it appears that the microbial population grew with the same rate as before the temperature shift-down. In Fig. 6, the growth curves were predicted as if the minimally processed endive was incubated at the same temperature as before the temperature shift (16°C, dotted lines) such that the number of pseudomonads were in close agreement with the assumed time–temperature profile predictions. Apparently, the new incubation temperature had no influence on the growth rate of the intrinsic microbial flora and the rule of additivity (McMeekin et al. 1993) was no longer valid for the growth of the number of pseudomonads on minimally processed endive stored under dynamic temperature conditions. In this case, it was not possible to predict the intrinsic microflora under changing temperature conditions.

A possible explanation for this observation could be found in a modified vegetable tissue. During sampling in the second week of the experiment, the appearance of the minimally processed endive was not acceptable because some of the pieces turned completely black. The leaves lost water as indicated by the marked drip at the bottom of the container. Possibly, the endive was unacceptable such that the growth of the micro-organisms was no longer controlled by the incubation temperature but by a complete breakdown of the

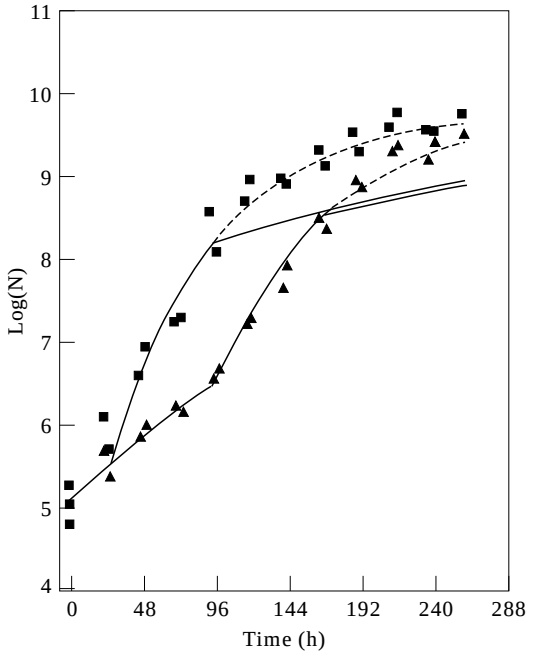

Figure 6. Predicted growth curves of the number of pseudomonads using the experimental time–temperature profile (—) and the assumed time–temperature profile (- - -) (experiment E). (■) 4-16-4-4°C, (■) 4-4-16-4°C.

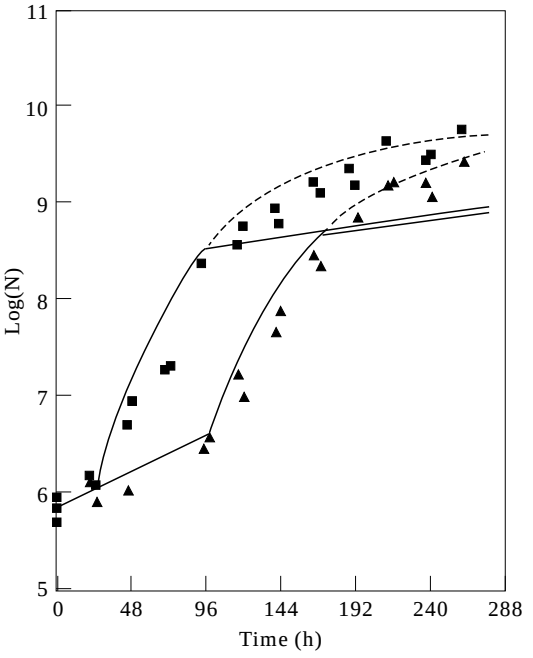

Figure 7. Observed and predicted growth data of the mesophilic aerobic count using the experimental time–temperature profile (—) and the assumed time–temperature profile (- - -) (experiment E). (■) 4-16-4-4°C, (▲) 4-4-16-4°C.

vegetable tissue. It should also be noted that the temperature shift occurred when the population density reached more than 8·5 log(cfu g^{-1}) and the samples would have been rejected for consumption.

Modelling growth of the Mesophilic Aerobic count under dynamic temperature conditions

Next to the number of pseudomonads, the MAC was enumerated during experiment E. Fig. 7 shows the observed and predicted MAC using the dynamic model for restricted growth in combination with the Arrhenius temperature dependence of the growth rate constant (Table 5). The MAC was underestimated after the temperature shift-down. Assuming the minimally processed endive would have been incubated at the temperature before the shift-down (approximately 16°C), the predicted growth curve (dotted line) was in better agreement with the observed MAC.

Conclusions

We can conclude that, given the initial microbial count and the environmental storage conditions, the number of pseudomonads and the MAC can be predicted under isothermal and constant carbon dioxide conditions. The temperature dependence of the growth rate constant was independent of the raw materials used. The number of pseudomonads and the psychrotrophic, Gram-negative bacteria were almost equal in a temperature range from 4–16°C. The growth of the pseudomonads population and the MAC was retarded by the gas composition of the headspace, while the lactic acid population was not affected.

The predictive performance of the new combined temperature growth was also evaluated. The explicit model for restricted growth was differentiated with respect to time. The microbial evolution of minimally processed endive stored under dynamic tem-

perature conditions was predicted using the model parameters obtained under constant temperature conditions. From this study, it was concluded that the rule of additivity was not longer valid. After the second temperature shift, the number of pseudomonads and the MAC were underestimated. It was suggested that this phenomenon could be related to the complete breakdown of the vegetable tissue. The release of additional nutrient was an important growth controlling factor. In contrast, a temperature change before the intrinsic microflora of the cut endive reached unacceptable levels was predicted fairly well. It was concluded that further research is necessary to investigate the observed deviation in predicted and counted microbial load.

A quantitative approach as illustrated here can be a helpful tool to industry in analysing the effect of storage conditions (temperature conditions and gas atmosphere) on the microbial quality of refrigerated foods. A potential application could be the analysis of the effect of temperature conditions as observed in different distribution chains.

Acknowledgements

This text presents research results of the Belgian Incentive Program 'Health hazards' initiated by the Belgian State—Prime Minister Office—Federal Office for Scientific, Technical and Cultural Affairs. The scientific responsibility is assumed by its authors. The authors thank the Federal Office for Scientific, Technical and Cultural Affairs for financial support.

References

Anon. (1988) Guide de bonnes pratiques hygiéniques concernant les produits végétaux prēts à l'emploi, dits de "IV" gamme. B.O.C.C.R.F. du 13 aoūt 1988.

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

Brackett, R. E. (1987) Microbiological consequences of minimally processed fruits and vegetables. *J. Food Quality* **10,** 195–206.

Carlin, F., Nguyen-The, C. and Varoquaux, P. (1990) La conservation des produits de la 4ème gamme, Industries Alimentaires et Agricoles, octobre 1990, 931–944.

Day, B. P. F. (1992) Guidelines for the good manufacturing and handling of modified atmosphere packed food products, CFDRA, Technical manual No 34, Chipping Campden, UK.

Denis, C. and Picoche, B. (1986) Microbiologie des légumes frais prédécoupés. *Industries Alimentaires et Agricoles* **103,** 547–553.

Duncan, D. W. and Nickerson, J. T. R. (1962) Effect of environmental conditions on the phase of adjustment of *Pseudomonas fragi. J. Appl. Microbiol.* **11,** 179–183.

Evans, J. A., Stanton, J. I., Russell, S. L. and James, J. S. (1991) Consumer handling of chilled foods: a survey of time and temperature conditions, pp. 102, Ministry of Agriculture, Fisheries and Food (MAFF) Publications, London.

George, S. M. and Lund, B. M. (1992) The effect of culture medium and aeration on growth of *Listeria monocytogenes* at pH 4·5, *Letters Appl. Microbiol.* **15,** 49–52.

Haralampu, S. G., Saguy, I. and Karel, M. (1985) Estimation of Arrhenius model parameters using three least squares method. *J. Food Process. Preserv.* **9,** 129–143.

Hudson, J. A. and Mott, S. J. (1993) Growth of *Listeria monocytogenes, Aeromonas hydrophila* and *Yersinia enterocolytica* on cooked beef under refrigeration and mild temperature abuse. *Food Microbiol.* **10,** 429–437.

Jarvis, B. (1989) *Statistical aspects of the microbiological analysis of foods. Progress in Industrial Microbiology.* Volume 21. Elsevier, Amsterdam.

Jason, A. C. (1983) A deterministic model for monophasic growth of batch cultures of bacteria, Antonie van Leeuwenhoek. *J. Microbiol. Serol.* **49,** 513–536.

Langston, S. W., Altman, N. S. and Hotchkiss, J. H. (1993) Within and between sample comparisons of Gompertz parameters for *Salmonella enteritidis* and aerobic plate counts in chicken stored in air and modified atmosphere. *Int. J. Food Microbiol.* **18,** 43–52.

Manvell, P. M. and Ackland, M. R. (1986) Rapid detection of microbial growth in vegetable salads at chill and abuse temperatures. *Food Microbiol.* **3,** 59–65.

Mackey, B. M. and Kerridge, A. L. (1988) The effect of incubation temperature and inoculum size on growth of Salmonellae in minced beef. *Int. J. Food Microbiol.* **6,** 57–65.

McMeekin, T. A., Olley, J. N., Ross, T. and Ratkowsky, D. A. (1993) Predictive Microbiology: Theory and Application, Research Studies Press, Taunton, Somerset, England.

Mossel, D. A. and Jacobs-Reitsma, W. F. (1990) Mikrobiologisch onderzoek van levensmiddelen. Strategie, principes en methoden, pp 303, P.C. Noordervliet bv. Zeist.

O'Beirne, D. (1990) Modified atmosphere packaging of fruits and vegetables, pp. 183–199, *In* T. R. Gormley (ed)., *Chilled foods. The state of the Art.* Elsevier Applied Sciences. London and New York.

Pamment, N. B., Hall, R. J. and Barford, J. P. (1978) Mathematical modeling of lag phases in microbial growth. *Biotechnol. and Bioeng.* **20,** 349–381.

Picoche, B. and Denis, C. (1988) Influence du conditionnement sur la conservation des légumes 4e gamma. I.A.A. Octobre, 925–928.

Ratkowsky, D. A., Olley, J., McMeekin, T. A. and Ball, A. (1982) Relationship between temperature and growth rate of bacterial cultures. *J. Bacteriol.* **149,** 1–5.

SAS Institute Inc. (1982) *SAS User's guide: Statistics,* 1982 Edition, pp. 584, Cary, NC: SAS Institute Inc.

Sinell, H. J. (1980) Packaging. Ch. 11. pp. 193. *In* J. H. Silliker, R. P. Elliot, A. C. Baird-Parker, F. L. Bryan, J. H. B. Christian, D. S. Clark, J. C. Olson Jr. and T. A. Roberts (ed.), *Microbial Ecology of Foods.* Vol. I. Academic Press, New York.

Van Impe, J. F., Nicolaï, B. M., Martens, T., De Baerdemaeker, J. and Vandewalle, J. (1992) Dynamic mathematical model to predict microbial growth and inactivation during food processing. *Appl. Environ. Microbiol.* **58,** 2901–2909.

Willocx, F., Hendrickx, M. and Tobback, P. (1994) A preliminary survey into the temperature conditions and residence time distribution of minimally processed MAP vegetables in Belgian retail display cabinets. *Int. J. Refrig.* **17,** 436–444.