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A Model to Predict Microbial Contamination of Blanched Spinach

E. Mayer-Miebach, B. Zanoni* and W. E. L. Spiess

E. Mayer-Miebach, W. E. L. Spiess: Institute of Process Engineering, Federal Research Centre for Nutrition, Engesserstr. 20, 76131 Karlsruhe (Germany)
B. Zanoni: DISTAM, Section of Food Technology, University of Milan, Via Celoria, 2, 20133 Milan (Italy)
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To predict the microbial concentration of blanched spinach a mathematical model was set up which takes microbial heat resistance into account. Two versions describe microbial counts of vegetative cells and of endospores in blanched spinach as a function of operating conditions during batch and continuous blanching. The predictive model was verified in several batch blanching experiments. Although based on simplified assumptions such as a first-order kinetics model and literature data for thermal inactivation, calculated values for microbial concentration on blanched spinach and in blanching water were similar to the experimental ones. The model can also be applied to a typical industrial continuous blanching treatment of spinach.

Keywords: blanching; dynamic modelling; food microbiology; spinach

Introduction

Some phenomena occurring during blanching such as microbial contamination have not been sufficiently explored. For example, as far as blanching of spinach in water before freezing is concerned, it is not clear why microbial concentration of blanched spinach is often much higher than predicted by models of thermal inactivation kinetics. Literature thermal inactivation kinetic data (1,2) have shown that vegetative cells are completely, and endospores only little inactivated during blanching of spinach (e.g. at 95 °C for 1 min). According to literature and industrial data (3) however, total aerobic microbial counts are about 10^4 cfu/g, sometimes even exceeding 10^6 cfu/g, and *Enterobacteriaceae* may reach 10^4 cfu/g. This discrepancy may depend on the temperature profiles of spinach during blanching. Raw spinach is loaded into the blancher in 'packages' of about 5–10 cm in height. The blanching temperature inside such 'packages' may be too low to inactivate vegetative microorganisms completely. Special microbial heat resistance should also be taken into account. Some authors (4,5) report microbial subpopulations which are resistant to heat as a result of tailing.

The aim of this work is to elucidate changes in microbial counts during blanching of spinach and to set up a computer model to predict the microbial concentration of blanched spinach and blanching water during both batch and continuous blanching in water.

*To whom correspondence should be addressed.

Materials and Methods

Materials

Fresh spinach was purchased from local markets on the day the experiments occurred and stored in a refrigerating chamber at 5 °C until use. All tests were carried out using spinach without stalk ends.

Microbial analyses were carried out to determine total counts of aerobic mesophilic bacteria, both vegetative cells and endospores. Spinach (100 g) was weighed aseptically, blended (Waring Commercial Blendor) with 400 mL of buffered peptone water (10 g casein peptone, 5 g sodium chloride, 3.569 g disodium hydrogenphosphate and 1.5 g potassium dihydrogenphosphate L^{-1}) and homogenized (Laborhomogenisator, Braun Melsungen, Germany). Aliquots of dilutions (100 μL) were spread-plated onto Plate Count Agar (PCA) (Tryptone Glucose Yeast Agar, pH 7.0) (Merck, Darmstadt, Germany) for determination of total aerobic mesophilic counts after incubation for 24 to 72 h at 30 °C.

Total activated endospore counts of raw spinach were determined in the homogenized sample after thermal inactivation of vegetative cells at 70 °C for 15 min in a thermostated water-bath (Haake, Karlsruhe, Germany). It was assumed that endospores were neither activated nor inactivated and that there was complete inactivation of vegetative cells (6). Methods and data processing are summarized in **Fig. 1**. Moisture of raw spinach (approx. 10 g) was determined gravimetrically at 105 °C.

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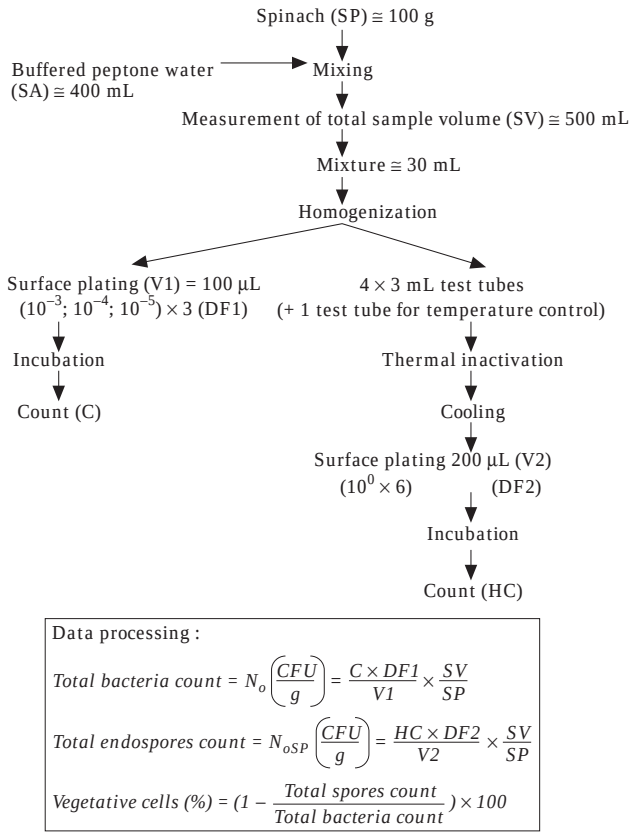

Fig. 1 Method and data processing of microbial analyses of raw spinach

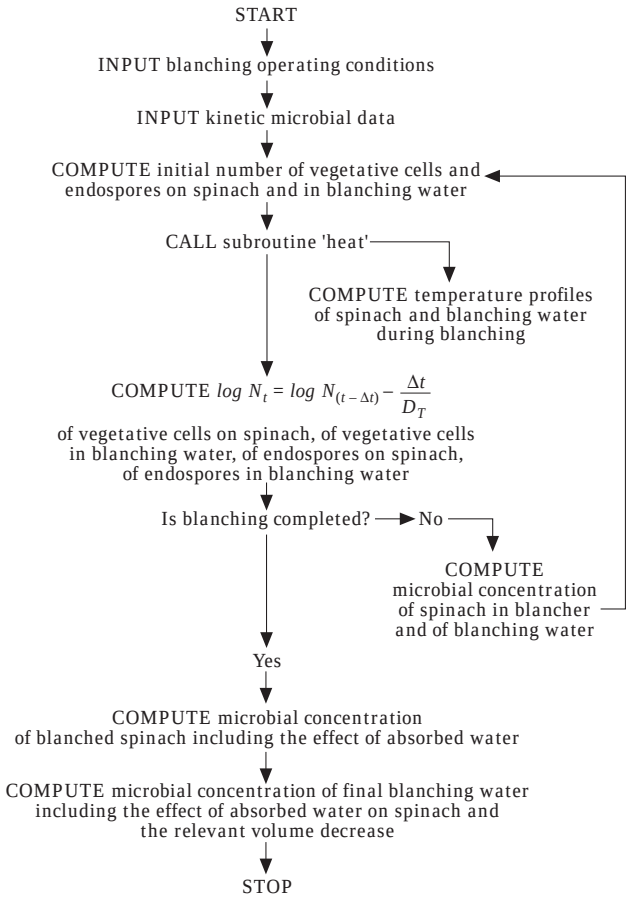

Fig. 2 Flow chart of batch programme

Phenomenological model

The microbial population of raw spinach is characterized by mixed vegetative cells and endospores. When raw spinach is placed into blanching water, some of the microorganisms remain at the spinach surface, while others move into the blanching water. Then, microorganisms on spinach and in blanching water may be inactivated following thermal inactivation kinetics. Vegetative cells and endospores show different kinetics. At the end of blanching, blanched spinach and blanching water are removed. The microbial concentration of blanched spinach depends on both surviving microorganisms at the surface and contamination of blanching water.

Mathematical models

Two predictive computer models of blanching were set up: the first describes microbial contamination in a batch blancher (i.e. batch programme), the second in a continuous blancher (i.e. continuous programme).

The computer flow chart of the batch programme is reported in **Fig. 2**. The batch programme predicts thermal inactivation of endospores and vegetative cells on spinach and in blanching water during batch blanching as a function of relevant temperature profiles. During this operation a batch of raw spinach is blanched in a stainless steel basket under given time and temperature conditions. Water is continuously heated by a thermostat. Both basket and spinach in the hot blanching water are heated as a result of combined heat transfer by convection–conduction. Internal resistance to conductive heat transfer in both the basket and spinach may be neglected (Biot number < 0.1). Heating of both basket and spinach results in cooling of blanching water and in combined convective–conductive–convective heat transfer from heat exchanger to blanching water. At the end of blanching the basket of blanched spinach is removed, fresh water may be added and the blancher is ready to start again. The batch programme may describe both one single and several successive blanching treatments with or without addition of fresh water.

The batch programme is based on the following assumptions: the blancher is shaken properly; a constant number of microorganisms, i.e. vegetative cells and endospores, is removed from the spinach surface; microorganisms are inactivated during blanching following first order kinetics; inactivation rate constants depend on the temperature, according to the Arrhenius approach; dormant endospores are not activated during blanching.

According to **Fig. 2**, equations describing microbial thermal inactivation kinetics were solved by the numerical integration approach. Temperature profiles were divided into short time intervals δt. At each time interval the temperature was considered to be constant. So, each interval represented a short thermal inactivation phase under isothermal conditions, and total

thermal inactivation was the sum of the individual thermal inactivation phases at δt.

Input data and values of the batch programme, including blanching operating conditions and kinetic microbial data, are reported in **Table 1**. Output data include absolute value and concentration of vegetative cells and endospores on spinach and in blanching water both during and after blanching.

The computer flow chart of the continuous programme is shown in **Fig. 3**. The continuous programme predicts thermal inactivation of endospores and vegetative cells on spinach and in blanching water for a typical industrial treatment as a function of relevant temperature profiles. During this operation batches of raw spinach are loaded into the blancher and immersed in the hot blanching water under given time–temperature conditions. When the blancher is in steady-state, spinach is unloaded in a continuous mode. As raw spinach in the blancher is moved by mechanical devices (blancher-belts), enhancing contact with hot blanching water, it is very difficult to describe temperature profiles of both spinach and blanching water during blanching. Experimental temperature profiles of spinach and blanching water were therefore applied to the continuous programme. The programme follows the microbial history of the total of blanching water and of one batch-like portion of spinach.

The continuous programme is based on the same assumptions as the batch programme plus the following ones in addition: microorganisms are continuously supplied by raw spinach following a linear trend; fresh water can be continuously added following a linear trend.

The continuous and the batch programme require different input data in terms of operating conditions and the same input data in terms of kinetic microbial data. Input data and values of the continuous programme are listed in **Table 1**. The continuous pro-

Table 1 Data and variables to solve the mathematical models

Variables	Data
Batch blanching operating conditions	
Number of blanching treatments	1 to 10
Temperature steps related to blanching time	up to 600
Duration of blanching (s)	60 to 600
Amount of spinach (kg)	0.03
Blanching water absorbed by dry spinach (mL/g)	3
Dry matter of raw spinach (g/kg)	100
Weight of basket (g)	394
Amount of blanching water (L)	1.9
Specific heat of water (J/g/°C)	4.187
Specific heat of spinach (J/g/°C)	3.934
Specific heat of basket (J/g/°C)	0.465
Overall heat transfer coefficient between blanching water and heat exchanger (W/°C)	12
Convective heat transfer coefficient between spinach and blanching water (W/°C)	30
Convective heat transfer coefficient between basket and blanching water (W/°C)	30
Temperature of raw spinach (°C)	25
Temperature of basket (°C)	25
Temperature of blanching water (°C)	up to 94.8
Temperature of media of heat exchanger (°C)	up to 95
Continuous blanching operating conditions	
Overall temperature steps related to blanching time	variable
Duration of blanching (s)	60 to 600
Temperature steps to reach steady-state related to blanching time	variable
Flux of spinach (kg/h)	15,000
Flux of fresh water (L/h)	15,000
Volume of blancher (L)	15,000
Blanching water absorbed by dry spinach (mL/g)	3
Dry matter of raw spinach (g/kg)	100
Kinetic microbial data	
Initial microbial concentration (cfu/g)	up to 10^8
Vegetative cell fraction of spinach (%)	99.98
Fraction of microorganisms adhering to spinach during blanching (%)	80
Integration time interval (s)	1
D value of vegetative cells (min)	0.3
D value of endospores (min)	2.4
Reference temperature of vegetative cells (°C)	60
Reference temperature of endospores (°C)	121
Z value of vegetative cells (°C)	8
Z value of endospores (°C)	7.9

gramme has the same output data as the batch programme.

Batch blanching tests

Our laboratory blanching equipment consisted of a cylindrical glass container (2 L volume) provided with a jacket, and of a stainless steel basket in which spinach was blanched. The container was filled with 1900 mL of sterilized salt solution (8.5 g sodium chloride per L). The solution, agitated by a magnetic stirrer, was heated to blanching temperature by a thermostated bath with ethylene glycol. The basket containing 30 g of raw spinach, aseptically weighed, was then placed into the container. The basket inside the container was moved upward and downward by an electric motor. The container was provided with a lid with two holes for the motor arm and a thermocouple to measure the temperature of the solution. The thermocouple was connected to a data acquisition and recording system interfaced to a PC. All blancher components (i.e. container, basket, etc.) were washed with ethanol and rinsed with sterilized water prior to use.

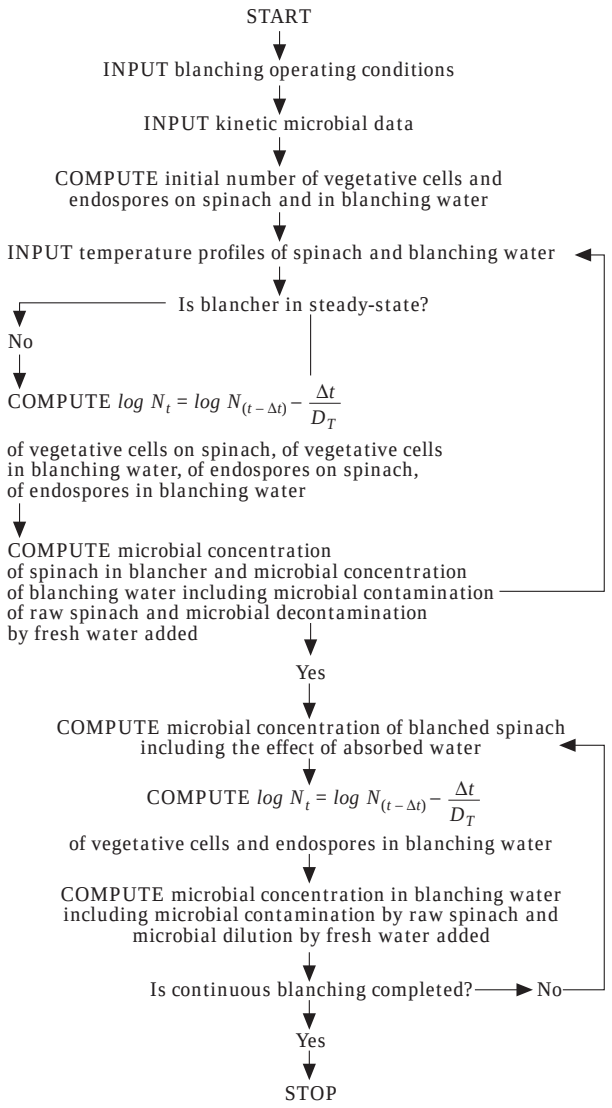

Fig. 3 Flow chart of continuous programme

Two series of blanching treatments were carried out. The first was to determine percent microorganisms adherent to spinach during blanching. Operating conditions were 'blanching' at 25 °C for 10 min, and 40 °C for 10 min. These conditions were chosen because they did not inactivate bacteria but showed their distribution between spinach and water.

Total aerobic mesophilic bacteria counts of blanched spinach and blanching water were determined by methods and data processing reported in **Fig. 4**.

The second series of blanching treatments was to study thermal inactivation kinetics of vegetative cells and endospores during blanching at 50 °C for 10 min, at 80 °C for 10 min, and at 95 °C for 1 min. Total aerobic mesophilic bacteria counts of blanched spinach and of blanching water were determined by methods and data processing reported in **Fig. 5**. In order to determine microbial counts of the blanching water, 10 mL aliquots were aseptically concentrated on a sterile filter (Microfil, Millipore GmbH, Germany) before incubation on PCA.

Continuous blanching tests

Five continuous blanching treatments were carried out in an industrial water-bath belt-blancher (designed by BFE industrial partner). Spinach flux and fresh water

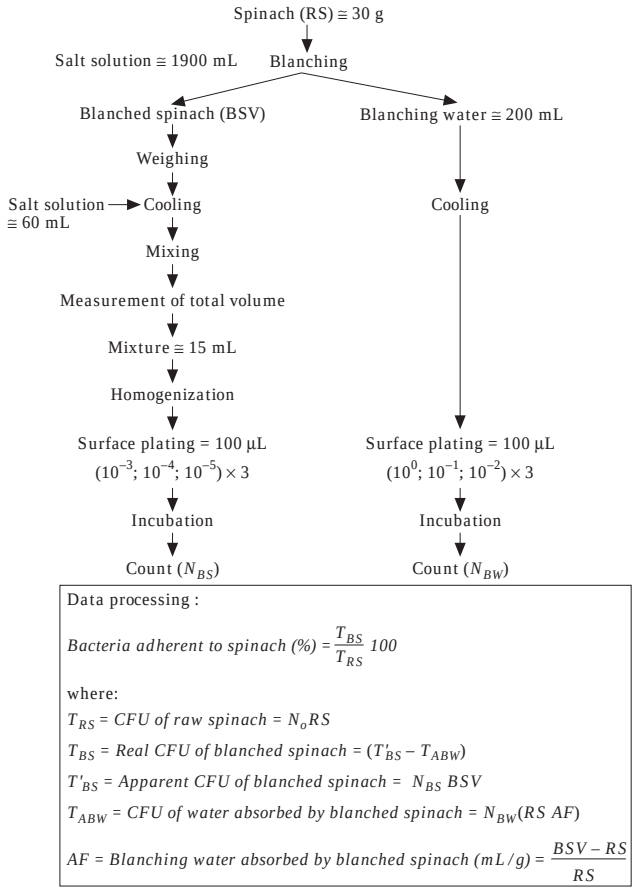

Fig. 4 Method and data processing of microbial analyses of spinach and blanching water in blanching tests at 25 and 40 °C

Table 2 Results from microbial analyses of batch blanching tests

Tests	No. (cfu/g)	Vegetative cells (%)	N_{BS} (cfu/g)	N_{BW} (cfu/mL)	Inactivated bacteria (%)	Inactivated bacteria (log N/N_0)	Bacteria adherent to spinach (%)	Contribution of blanching water (%)
25°C	7.79×10^6	99.97	3.18×10^6	12,300	n.s.	n.d.	83	n.d.
10 min	(11)[a]		(11)	(6)				
40°C	8.28×10^7	99.99	1.37×10^7	71,700	n.s.	n.d.	81	n.d.
10 min	(2)		(19)	(10)				
50°C	2.96×10^7	99.96	465,551	6300	97.00	–0.6	n.d.	0.4
10 min	(3)		(9)	(6)				
80°C	9.18×10^6	99.99	1021	≤13[b]	99.98	–3.7	n.d.	0.2
10 min	(5)		(20)					
	6.02×10^7	n.d.	5885	25	99.98	–3.8	n.d.	0.1
	(13)		(17)	(13)				
95°C	1.65×10^7	n.d.	2835	24	99.97	–3.5	n.d.	0.2
1 min	(6)		(6)	(11)				
	1.44×10^7	n.d.	2460	23	99.97	–3.5	n.d.	0.2
	(4)		(2)	(15)				

n.d. = not determined.
n.s. = not significant at $P < 0.05$.
[a] Experimental error is reported in brackets as percentage values of coefficient of variation.
[b] Detection limit.

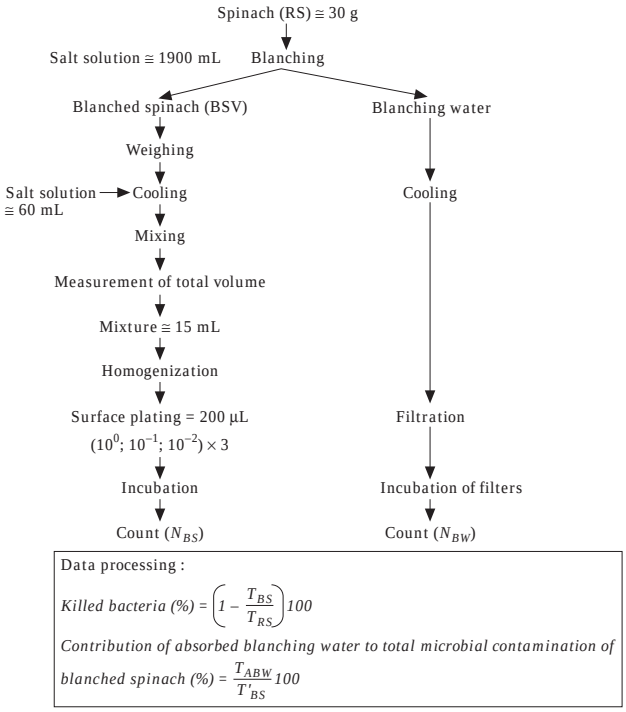

Fig. 5 Method and data processing of microbial analyses of spinach and blanching water in blanching tests at 50, 80 and 95 °C

flux were 15 t/h each, and blancher volume was 15 t. Industrial standard time–temperature conditions were 95 °C for 1 min. The temperature profile of batches of spinach during blanching was measured by a small electronic time–temperature probe (7), placed into 'packages' of spinach leaves. Data collected by the probe were transferred and processed by a PC.

Results and Discussion

Table 2 shows the results of microbial analyses of batch blanching tests: total bacteria counts of raw spinach (N_o) and percent vegetative cells of total bacteria counts, total bacteria counts of blanched spinach (N_{BS}) and of blanching water (N_{BW}), percent of completely inactivated bacteria after blanching, number of decimal reduction cycles after blanching, percent bacteria which remained adherent to spinach during blanching, and contribution of blanching water to the contamination of blanched spinach.

Vegetative cells constituted 99.98% of the total counts. Total counts (10^6–10^7 cfu/g) included a considerable amount of endospores (approx. 10^3–10^4 cfu/g). In accordance with literature data (1,2), blanching reduced microbial contamination by vegetative cells. After blanching at 80 °C for 10 min, or 95 °C for 1 min, 99.98% of total bacteria were inactivated; only endo-

Table 3 Comparison of experimental and calculated microbial data

Blanching	N_o (cfu/g)	Experimental N_{BS} (cfu/g)	Experimental N_{BW} (cfu/mL)	Calculated N_{BS} (cfu/g)	Calculated N_{BW} (cfu/mL)
50 °C, 10 min	2.96×10^7	465,551	6300	581,928	2830
80 °C, 10 min	9.18×10^6	1021	13	566	3
	6.02×10^6	5885	25	3710	19
95 °C, 1 min	1.65×10^7	2835	24	2033	10
	1.44×10^7	2460	23	1774	9

Experimental error is reported in **Table 2** as percentage values of coefficient of variation.

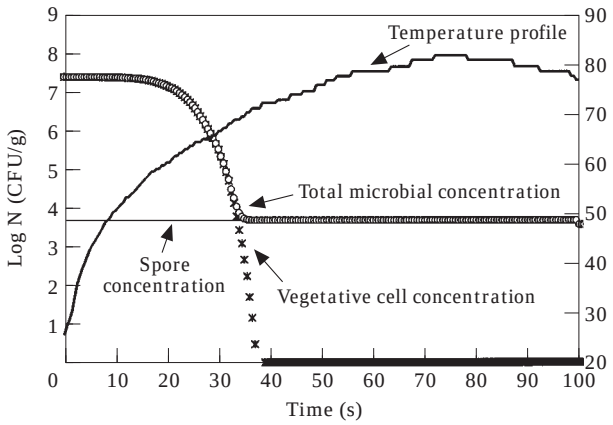

Fig. 6 Predicted results of a continuous blanching treatment: blancher functioning in a continuous mode for 100 s. (×) = vegetative cell concentration; (○) = total microbial concentration; (—) = spore concentration

spores survived. Blanching caused about four decimal reduction cycles of total bacteria counts. It is worth noting that blanching at 50 °C for 10 min inactivated 97% of total bacteria (i.e. about one decimal reduction).

The first series of blanching tests has shown that about 80% of bacteria were adhering to spinach. The second series has shown that the contribution of blanching water to contamination of blanched spinach was very low during one single batch blanching treatment.

Assuming that the total aerobic mesophilic bacteria comprised both vegetative cells and endospores, literature values (1) for the relevant thermal kinetic parameters D and z were used (**Table 1**). Subpopulation 1, i.e. vegetative cells, was characterized by literature thermal death kinetic data for *Pseudomonas*, and subpopulation 2, i.e. endospores, was characterized by literature thermal death kinetic data for the genus *Bacillus*. A comparison between experimental and calculated data is shown in **Table 3**. Calculated results are similar to the experimental ones. Although the predictive model was based on simplified assumptions, such as use of the first order kinetics model and of literature kinetic data, the batch programme was able to predict microbial concentration during batch blanching.

The present predictive model of blanching was useful to describe microbial thermal death kinetics during an industrial continuous blanching treatment of spinach. **Figure 6** shows predicted microbial concentration and experimental temperature profile of spinach during a continuous blanching treatment, where industrial standard time–temperature conditions were 95 °C for 1 min.

The temperature profile of spinach showed two characteristics: the holding time of batches of spinach exceeded 60 s (100 s on average), and the temperature of spinach did not reach that of blanching water (95 °C) immediately; spinach was heated gradually up to max. 80 °C. This showed that the operating conditions chosen for blanching did not correspond to those prevailing in practice.

The 'packages' of spinach leaves produced a significant resistance to heat transfer. However, this industrial blanching treatment was able to inactivate all vegetative cells, although the temperature of spinach did not reach 95 °C. This occurred in a range of 60 to 75 °C. The predicted microbial contamination of blanched spinach was approx. 4000 cfu/g, corresponding to the amount of endospores not inactivated during blanching.

Figure 7 shows the predicted total microbial concentration of blanched spinach and blanching water after treatment for 10 min in a continuous blancher. The programme was able to describe both thermal inactivation kinetics of microorganisms during blanching before steady-state (from 0 to 100 s), and (> 100 s) the microbial concentrations of the various batches of blanched spinach at the plant outlet, which had the same thermal history and, consequently, the same microbial inactivation kinetics as spinach in steady-state. The sharp, small decrease in concentration after 100 s was due to dilution of blanched spinach as a result of the water absorbed at the plant outlet.

With regard to blanching water, the programme was able to describe the trend of microbial concentration during blanching as a function of thermal inactivation kinetics, addition of fresh spinach, removal of blanched spinach and flux of fresh water. An increase in microbial concentration was observed which tended to reach an asymptote. In our case, the increase was about three logarithmic cycles. The asymptote value depended on the extent of microbial inactivation, i.e. on the flux of fresh spinach and the extent of microbial contamination; on the blanching time and on the temperature of the blanching water; and on the volume of blancher and on the flux of fresh water. It is evident that a high increase in microbial concentration of blanching water may result in recontamination of blanched spinach at the blancher outlet by the blanching water.

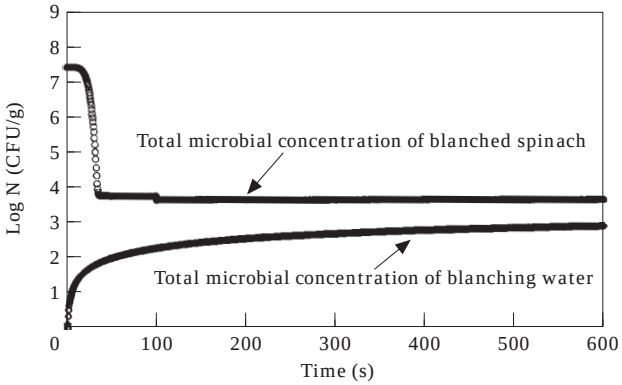

Fig. 7 Predicted results of a continuous blanching treatment: blancher functioning in a continuous mode for 10 min

Conclusions

The model was able to predict the microbial inactivation of bacteria, both vegetative cells and endospores, of spinach and water during batch blanching. During continuous blanching, the model was able to predict a

microbial contamination of blanched spinach of 10^4 cfu/g. However, total counts after blanching sometimes exceed 10^6 cfu/g, as industrial data indicate (3). This discrepancy is still unclear. In order to verify whether it depends on recontamination after blanching or on heat resistant microbial subpopulations, microbial analyses of raw and blanched spinach are presently being carried out in an industrial continuous blanching plant.

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