The combined effect of cooking and storing temperatures on the quantities of aerobic bacteria in cooked sausage

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SUMMARY

A 'plate type' temperature gradient incubator (Gradiplate[R], Biodata Ltd., Helsinki) was used to expose raw sausage mater to 81 different combinations of cooking (9 levels in the range of 54-69°C) and cold storage temperatures (9 levels in the ran of 1-11°C) according to a 9^2 factorial design. The numbers of naturally-present aerobic, coliform and lactic acid bacteria in t sausage material were determined after these two sequential incubations. The bacterial counts were plotted against the corresponding cooking and cold storage temperatures to give 3-dimensional 'maps'. Furthermore a six-order polynomial mod was fitted to the experimental data.

In general low bacterial counts corresponded with high cooking temperatures and low storage temperatures, but the overa pattern of growth was not readily predictable. Therefore the 3-dimensional maps can be used as a model to predict the probable numbers of bacteria in cooked and cold stored sausages. With the help of a polynomial model the combined effect cooking and cold storage temperatures on quantities of coliform and lactic acid bacteria was discovered.

This temperature mapping study also confirms that avoidance of postcookery recontamination provides the method of cho for improving the microbiological quality of cooked sausages.

INTRODUCTION

In food microbiology much effort has been directed at defining limiting values for bacterial growth. With few exceptions li Cl. botulinum influence of single factors, such as temperature, NaCl, pH, a_w on the growth of organisms have been studied. predictive food microbiology knowledge of combinations of these factors and their growth limiting values need to be studied

Two-dimensional gradient plates have been used for examining simultaneous and interactive effects of two chemical variab on microbial growth (WINPENNY et al. 1984, McCLURE et al. 1986, McCLURE et al. 1987). This work presents a two-dimensional procedure in which food is used as bacterial habitat for detecting interactive effects of heat process and cold storage temperatures.

MATERIALS AND METHODS

A plate type temperature gradient incubator (Gradiplate[R], Biodata Ltd., Helsinki) was used to expose raw sausage materia different combinations of cooking and cold storage temperatures. A cuvette with 9x9 separated wells was made to fit the temperature gradient incubator.

Samples of raw grill sausage material were collected from the stuffing equipment of a Finnish meat plant. The 81 wells of cuvette were able to take 0.5 grams of raw sausage material each. The sterile cuvette was covered with a tight glass lid to av both drying and contaminations of the sample material.

After filling the cuvette was first incubated 30 minutes in the range 54-69°C simulating sausage cooking. Then it was turne through an angle of 90 degrees. The incubation was continued for 14 days in the cold range (1-11°C). Thus each of the 81 w got a different temperature combination. The incubations took place under aerobic conditions. The gradient temperatures w checked with a calibrated data logger (Grant Squirrel SQ 1203, Grant Instruments Ltd., Cambridge) with K type thermocou placed in the sausage material.

The naturally-present aerobic, coliform and lactic acid bacteria in raw sausage material were enumerated with agar dip sli (Orion Diagnostica Ltd., Espoo). After 14 days cold incubation 81 sausage cubics were aseptically picked up from the wells homogenized and diluted in buffered peptone water (Merck). The agar dip slides were inoculated by dipping them into dil

10^{-3}. Aerobic bacteria were cultivated on APC-agar (Hygicult TPC), 20°C 72 h, coliforms on VRB-agar (Hygicult E), 37°C 24 h, and lactic acid bacteria on Rogosa-agar, pH 5,5 (Dentocult LB) anaerobically 20°C 5 days. The logarithmic bacterial counts were plotted against the corresponding cooking and cold storage temperatures.

The experiment was carried out according to 9^2 factorial design (BOX et al. 1956). Cooking and cold storage temperatures varied both in 9 levels and their all possible combinations were tested. Because both variables affected independently and orthogonally in the experiment, their combined as well as their straight effects on bacterial counts could be discovered.

Polynomial models were used to describe the mathematical relationship between the detected bacterial counts and cooking and cold storage temperatures. Up to sixth-order polynomials were fitted to the experimental data and the regression coefficients were determined. The normal probability plot of standardized regression coefficients was used to evaluate the experimental error as well as to find out the real effects of coefficients. In the plot nonsignificant coefficients lay on a straight line but significant fall off (BOX et al. 1978). Statistical analysis were combuted with PC-MATLAB 3.5a (1989).

RESULTS AND DISCUSSION

In general low bacterial counts corresponded with high cooking temperatures and low storage temperatures, but the overall pattern of growth was not readily predictable. The maps of aerobic bacterial counts (Figs 1a and 1b) revealed unexpectedly high numbers of bacteria in 4 independent samples, which formed a kind of isolate. In general the aerobic map was difficult to interpret, because of numerous 'small growth peeks' (Fig 1a), which interfered polynomial curve fittings, but we failed to predict the isolate, too.

Figures 1c and 1d show the corresponding maps of coliforms and lactic acid bacteria. Even with the mildest combination (54°C+11°C) the quantity of lactic acid bacteria did not exceed the limit $1x10^6$ cfu/g, which was set for heavy growth. In comparison with lactic acid bacteria coliforms seemed to tolerate slightly higher temperatures and/or were able to grow faster in the sausage medium under aerobic conditions. While recontamination of sausage samples after cooking was prohibited in this experimental procedure, the numbers of all examined bacteria were after 14 days cold storage still near or below the detection level $5x10^4$ cfu/g when cooking temperature was 69°C (Table 1).

Table 1. Examples of logarithmic numbers of aerobic bacteria (APC), coliform (COLI) and lactic acid bacteria (LAB) detected in sausage samples during the experiment.

SAMPLE COLLECTION	LOG NUMBER OF BACTERIA/g		
	APC	COLI	LAB
in the beginning	7.87	4.80	7.72
after cooking (69°C)	4.20	<2.0	<2.0
after cooking (54°C)	6.40	4.80	3.78
after cooking (69°C) * cold storage (1°C)	<4.7	<4.7	<4.7
after cooking (54°C) * cold storage (11°C)	8.00	8.00	6.00

The temperature check-ups showed that the gradient temperatures differed +/-0.6°C from the theoretical values calculated according to NIEMELÄ et al. (1990). It also took 15 minutes before the set cooking temperatures were stabilized.

Figure 2a reveals the normal probability plot of regression coefficients calculated from the coliform data. The most important coeffients were C, C^2, S, C*S, respectively (C=cooking temperature, S=cold storage temperature, C*S=1st degree combined effect of C and S). In the case of coliforms the goodness of fit of polynomial model was $R^2=0.8753$. With lactic acid bacteria $R^2=0.8024$ was achieved with a model containing coefficients C, C*S, S, C^2, C^2*S (Fig. 2b). Figures 3a and 3b show the two-dimensional contour maps of optimum responses after model fittings.

CONCLUSIONS

A 'plate type' temperature gradient incubator can be used to examine the combined effect of food processing and cold storage

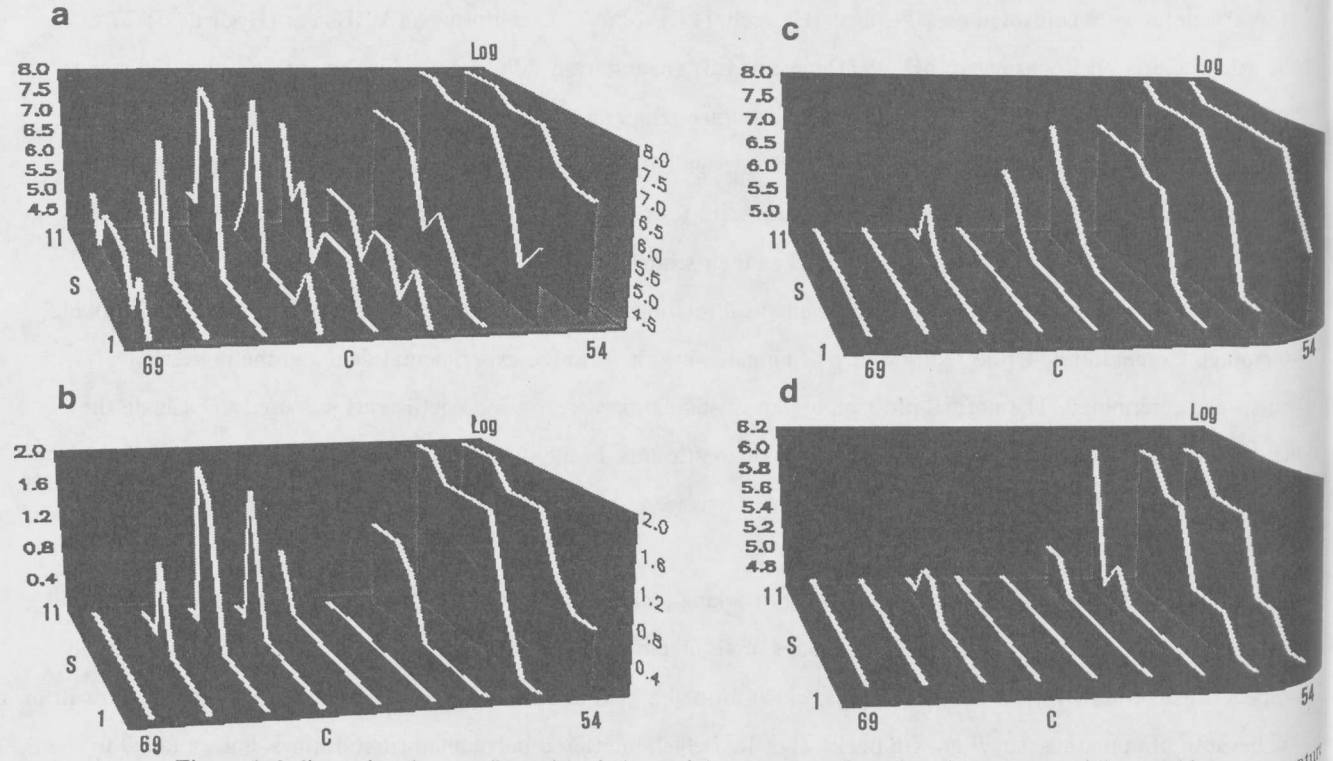
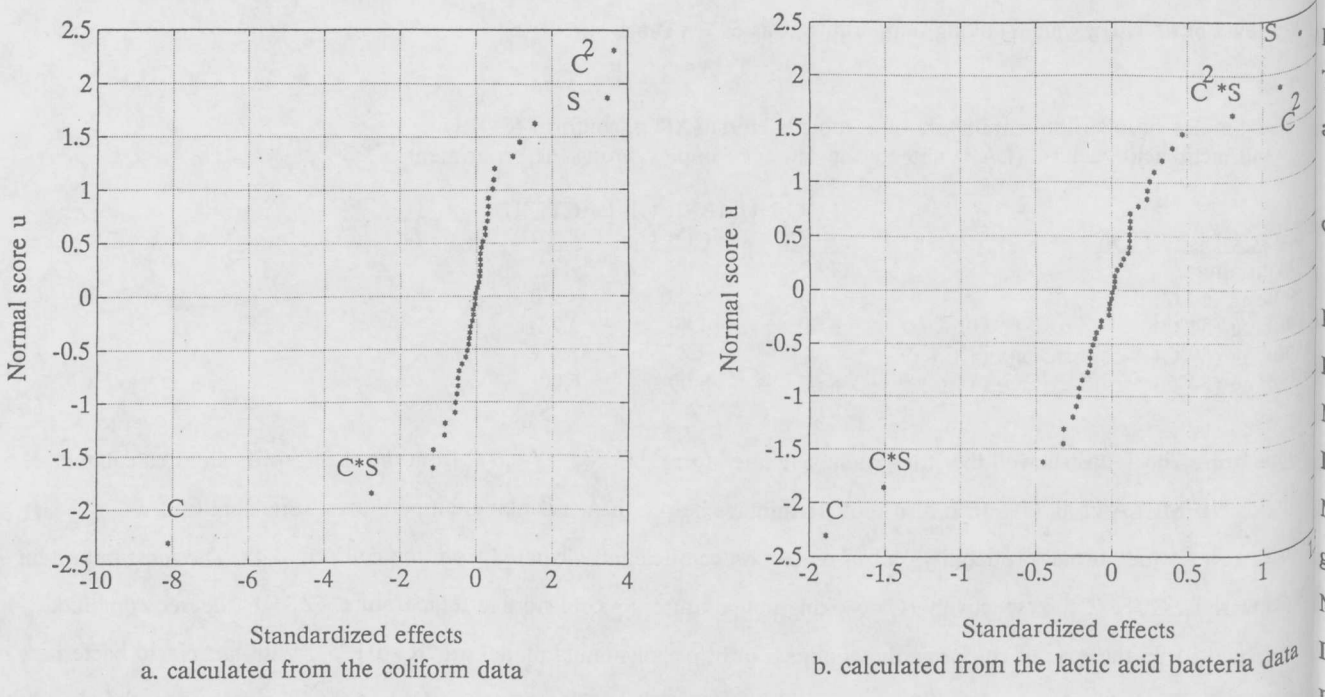


Figure 1. 3-dimensional maps. Logarithmic bacterial counts are plotted against corresponding cooking temperatur (C-axis, 54-69°C) and cold storage temperatures (S-axis, 1-11°C).

a. Aerobic bacteria
b. Aerobic bacteria, focused on areas of heavy growth (original log numbers reduced by 6)
c. Coliform bacteria
d. Lactic acid bacteria

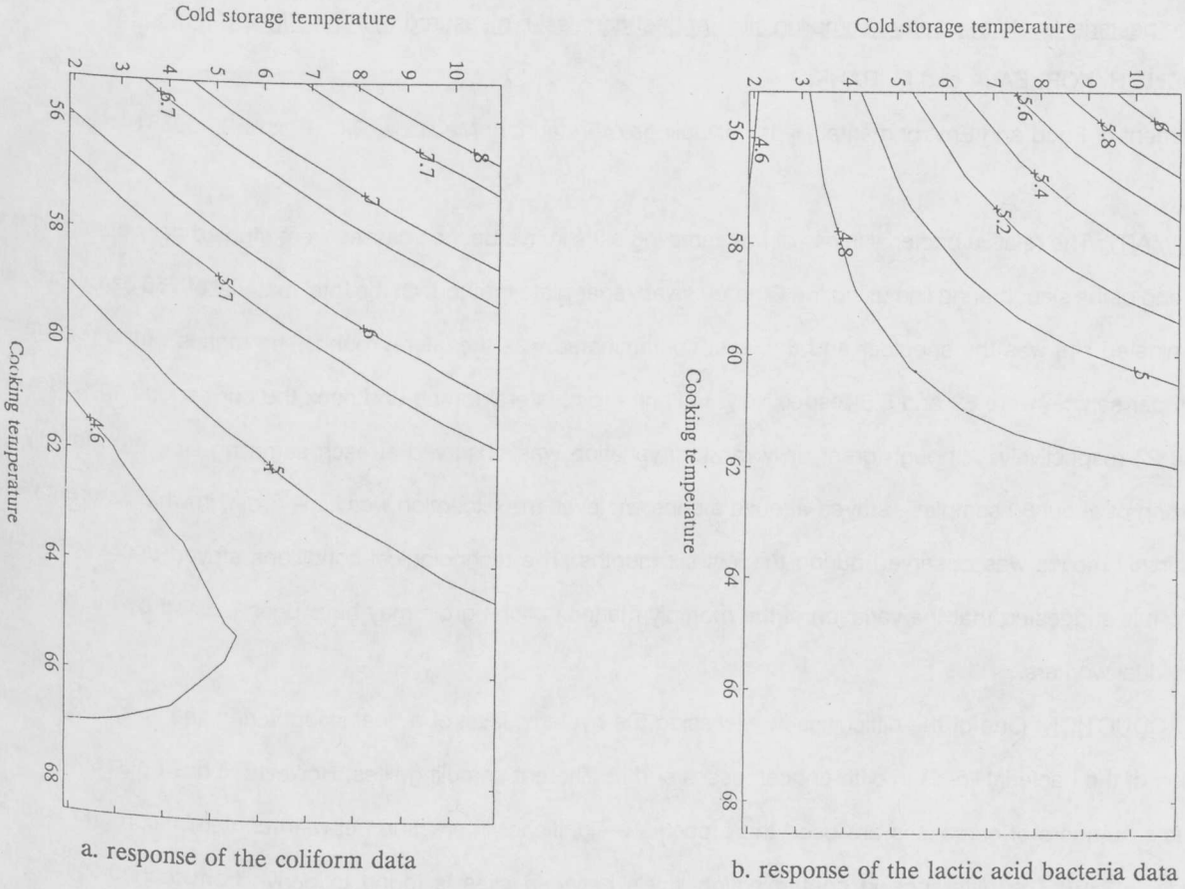


a. calculated from the coliform data

b. calculated from the lactic acid bacteria data

Figure 2. The normal probability plots of regression coefficients.

C = cooking temperature
S = cold storage temperature
C * S = combined effect of C and S



a. response of the coliform data

b. response of the lactic acid bacteria data

Figure 3. 2-dimensional contour maps of optimum responses after polynomial model fittings.

temperatures. General trends and interesting abnormalities in thermal responses of bacteria in food can be discovered by this method. The use of homogenous food itself as a culture medium offers for example the possibility of testing efficacy of food preservatives in natural environments versus various heat treaments and cold storage temperatures over relatively wide ranges. This method bound together with Response Surface Methology formulating the data into contour maps minimizes the overall amount of laboratory work to construct a predictive model.

Besides this temperature mapping study confirms that avoidance of postcookery recontamination provides the method of choice for improving the microbiological quality of cooked sausages.

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