

## PROCESS CONTROL IN THE PRODUCTION OF FERMENTED SAUSAGES

R. SKJELKVÅLE, P.G. FYHN, E. SLINDE and T. HØYEM

Norwegian Food Research Institute, P.O.Box 50, N-1432 Ås-NLH, NORWAY

## INTRODUCTION

COMPUTERIZED process control of the dry sausage fermentation process presents several intriguing problems. The most prominent aspects to be taken into consideration are the recipe, temperature, relative humidity and the nature and use of the starter culture. The fermentation takes place in a closed container which leaves small or no possibilities for monitoring of important parameters like bacterial counts and water activity. On the other hand, the only means of influencing the fermentation seem to be through control of temperature and relative humidity.

We have been studying various aspects of dry sausage fermentation in order to reach a mathematical model that can be used in computerized control of the fermentation process. So far the work has been restricted to the stage where pH reaches its minimum. The drying process will later be included in the model.

In the present study, we have investigated the interrelationship between bacterial counts, carbohydrate consumption, lactic acid production and development of pH.

## MATERIALS AND METHODS

SAUSAGES used for the experiments were made according to one of the standard recipes used in the Norwegian meat industry, with 0.6% carbohydrate as glucose (0.4%) and dextrin (0.2%). The minced meat was stuffed in 70 mm fibrous casings and made to a dimension of 12 cm length (400 g). The starter culture used was a homofermentative lactobacillus isolated from Duplo-ferment 66 (Rudolf Müller & Co., Giessen, W.Germany), grown for 20 hours on MRS medium (de Man *et al.*, 1960), and suspended in peptone water to give  $4 \cdot 10^6$  bacteria per gram of minced meat. The sausages were ripened at 25°C and 90% R.H. Samples were taken at 12 hr intervals and analyzed on dry weight basis for viable counts, carbohydrate, and D- and L-lactic acid. Loss in weight and pH were also recorded.

VIABLE COUNTS were determined by plate counting on blood agar (25°C in 48 hrs).

CARBOHYDRATE was assayed as glucose using the Boehringer Mannheim food analysis kit, cat. no. 139106.

LACTIC ACID (D- and L-) was measured enzymatically by the use of the Boehringer Mannheim food analysis kit (cat. no. 139084 for L-lactic acid, the L-lactate dehydrogenase being replaced by D-lactate dehydrogenase when D-lactic acid was assayed).

pH was determined in a suspension of 5 g of sausage material in 30 ml of dist. water. pH was also measured "continuously" during the fermentation process by an inserted Ingold glass electrode based on a "switch on/off" program with 1 hr intervals to avoid errors due to changes in the potential over the glass electrode membran.

THE MATHEMATICAL MODEL used for the simulation purpose is based on four state variables: bacterial growth, carbohydrate, lactic acid and pH. In order to describe the dynamic process for these, the following models have been found to give the best description of our production conditions:

- a. Modelling of bacterial growth (Constantinides *et al.*, 1970)

$$dx_1/dt = \dot{x}_1 = \mu_{\max} \frac{x_1}{1 + k_s/x_2 + x_2/k_s}$$

where;  $\mu_{\max}$ =max., specific growth coefficient,  $k_s$ =saturation constant,  $k_i$ =inhibition constant;  $x_1$ =number of bacteria, and  $x_2$ =carbohydrate concentration.

- b. Modelling of carbohydrate concentration (glucose) (Andreyeva and Biryukov, 1973)

$$ds/dt = \dot{s} = \dot{x}_2 = -1/Y \cdot S \cdot C$$

where: S=substrate concentration, C=dry weight of biomass,  $\mu$ =specific growth coefficient; Y=yield coefficient.

c. Modelling of lactic acid concentration (Hansen and Tsao, 1972)

$$dx_3/dt = \dot{x}_3 = b_1 \cdot S \cdot C$$

where:  $b_1$  = rate constant.

d. Modelling of pH

$\text{pH} = -\log [\text{H}_3\text{O}^+]$  is transformed to  
 $\text{pH} = y = -\log k_1(x_3 + k_2)$

where:  $k_1$  and  $k_2$  are rate constants.

The symbols of equations a, b, c and d are rewritten as shown:

$$(i) \quad \dot{x}_1 = \frac{P_1 \cdot u_1 \cdot x_1}{1 + P_2/x_2 + P_3 \cdot x_2}$$

$$(ii) \quad \dot{x}_2 = -P_4 \cdot x_1 \cdot x_2$$

$$(iii) \quad \dot{x}_3 = -P_5 \cdot \dot{x}_2$$

$$(iv) \quad y = -\log P_6 (x_3 + P_7)$$

where  $P_1$ - $P_7$  are constant terms.

$u_1$  is control variable (temperature) and  $y$  is output variable (pH).

Based on these four equations, simulation was performed on a Nord-10/S computer (Norsk Data, Oslo, Norway).

$P_1$ - $P_7$  were found by studying the four state variables ( $x_1$ ,  $x_2$ ,  $x_3$  and  $y$ ) from 14 production experiments and represent average figures for the experiments.

## RESULTS

CARBOHYDRATE consumption, lactic acid production, and pH development, together with viable counts in a typical production experiment are shown in Fig. 1.

While the pH is intercorrelated to carbohydrate consumption and lactic acid formation, no correlation has been found between the three parameters and viable counts (Fig. 2).

The absence of correlation between viable counts and the other parameters can in part be explained by the ability of non-dividing cells to metabolize carbohydrates. This was shown in an experiment where washed lactobacilli in buffer, pH 7.0, failed to propagate, as judged by the absorbancy at 620 nm, while lactic acid was produced when glucose was present. In the absence of glucose no detectable amounts of lactic acid was produced (Fig. 3).

The fit between experiments and the model can be improved by using equation (i)-(iv) with the so-called Kalman filter (Tzafestas and Nightingale, 1968).

The parameters  $P_1$ - $P_7$  were estimated for 14 production experiments and the average results of these experiments are shown in Fig. 4.

## DISCUSSION

The successful computerized process control of dry sausage fermentation requires a thorough knowledge of the microbiological and biochemical factors governing the process. In general it can be stated that a rapid fall in pH to 4.8-5.0 promotes the desired characteristics of product colour and texture. The pH is dependent on the formation of acids during fermentation and the interaction of these acids with meat proteins (Andersen & Ten Cate 1965). We have confirmed the observation made by Demeyer & Vandekerckhove (1979) that the pH is significantly correlated to lactate concentration. In addition, we have found correlation between the carbohydrate and total lactate concentrations, and it seems to exist a simple relationship between these three parameters. In spite of the fact that most of the dramatic changes seen during the fermentation process in dry sausages are due to the propagation of lactic acid bacteria, no connection whatsoever could be drawn between viable counts and the carbohydrate or lactic acid concentrations. Therefore, viable counts have to be less regarded in a mathematical model describing the fermentation process. The growth curve follows the gene-

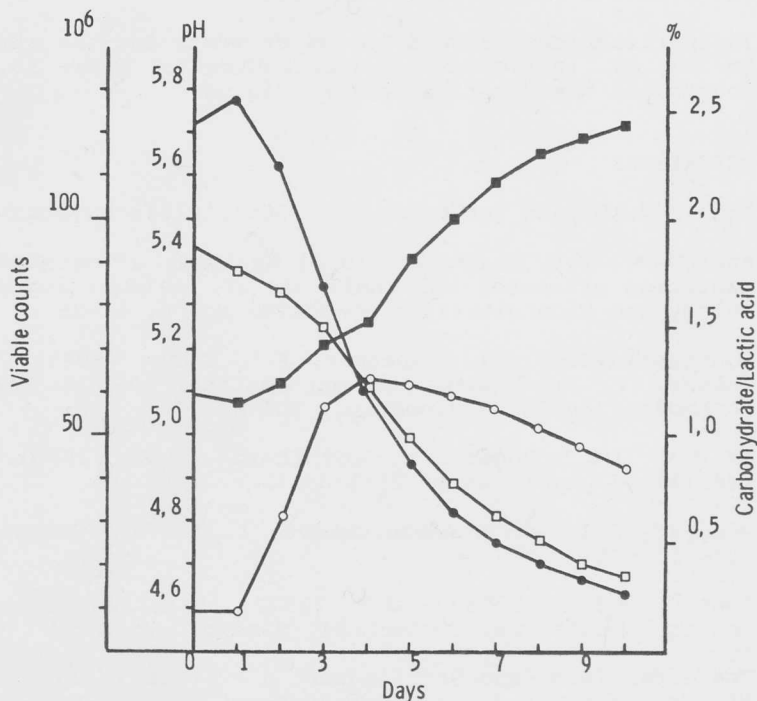


Fig. 1  
 Viable counts (o-o), pH (●-●), carbohydrate (□-□), and lactic acid (■-■) levels during the fermentation process as described in Materials and Methods.

rally observed pattern: an exponential growth phase followed by the stationary phase, and the death phase. From our studies it can be concluded that cells, not able of division, are capable of converting glucose to lactic acid, even though the turnover rate might be different from the one in dividing cells.

The correlations between the experiments and the simulated models are acceptable with regard to the pH, lactic acid and carbohydrate. There is, however, no correlation between viable counts and the other variables (Fig. 4).

#### REFERENCES

- Andersen, G. and Ten Cate, L. (1965). *Fleischwirtsch.* **45**, 599-606
- Andreyeva, V.V. Biryukov (1973) Analysis of mathematical models of the effect of pH on fermentation processes and their use for calculating optimal fermentation conditions. *Biotechnology and Bioengineering Symposium No. 4*, 61-76.
- Constantinides, J.L., Spencer, E.L. Gaden (1970). Optimization of batch fermentation processes. I. Development of mathematical models for batch penicillin fermentations. *Biotechnology and Bioengineering*, Vol XII.
- de Man, J.C., Rogosa, M. and Sharpe, M.E. (1960). A medium for the cultivation of lactobacilli. *J. Appl. Bact.* **23**:130-135
- Demeyer, D.I. and Vandekerckhove, P. (1979). Compounds determining pH in dry sausage. *Meat Sci.* 161-167
- Hansen, T.P. and Tsao, G.T. (1972). Kinetics of the lactic acid fermentation in batch and continuous cultures. *Biotechnol. Bioeng.*, Vol. XIV
- Tzafestas, S.G. and Nightingale, J.M. (1968). Optimal filtering, smoothing and prediction in linear distributed parameter systems. *Proc. IEE*, Vol. 15, No. 8, p.1207

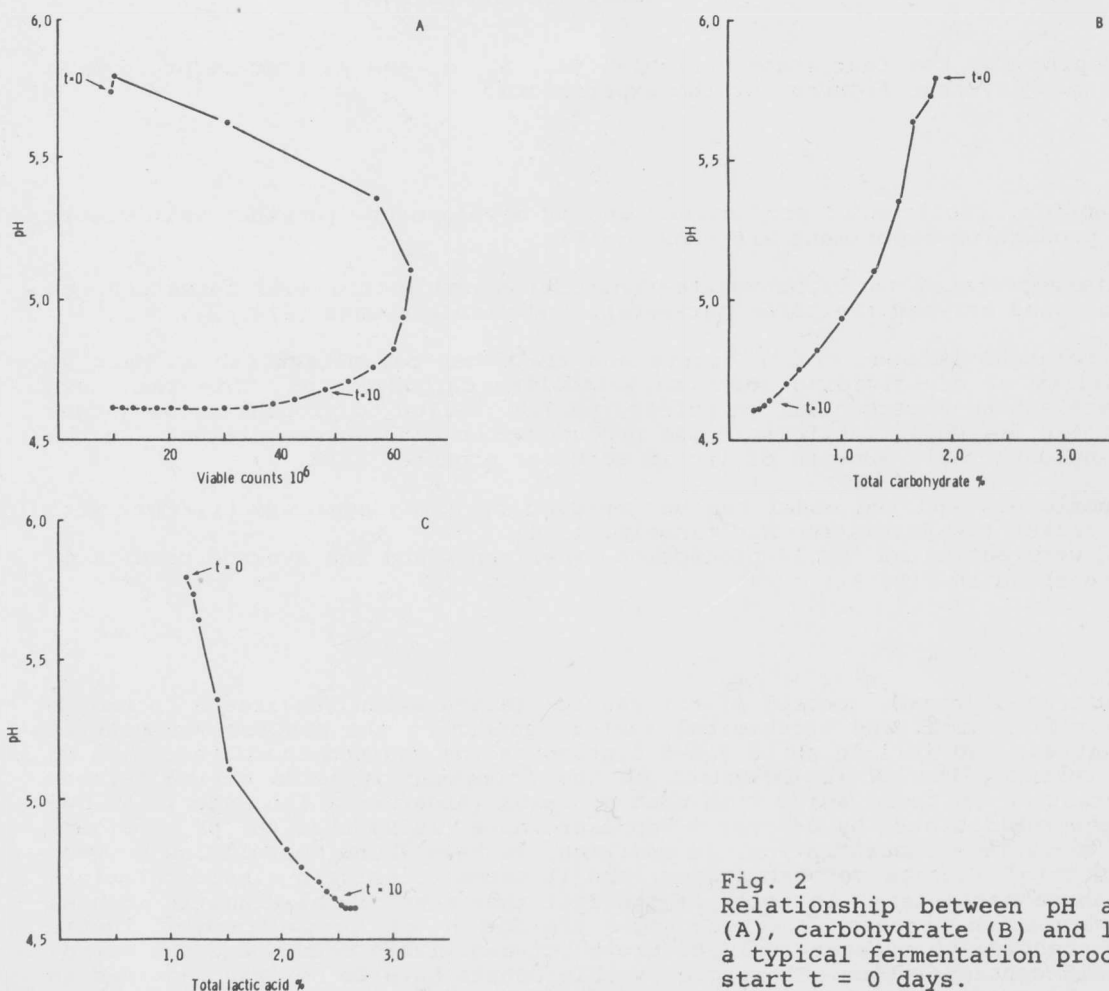


Fig. 2  
Relationship between pH and viable counts (A), carbohydrate (B) and lactic acid (C) in a typical fermentation process. Fermentation start  $t = 0$  days.

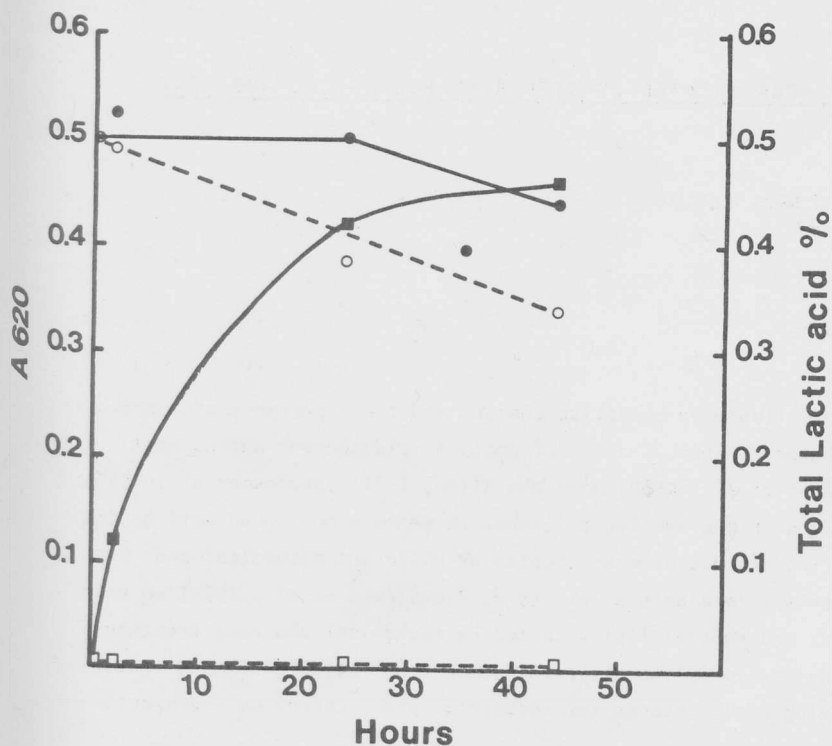


Fig. 3  
Metabolizing ability of non-dividing lactobacilli. Absorbancy measurements at 620 nm with glucose (●-●) and without glucose (control) (○-○). Total lactic acid production in the presence (■-■) and absence (□-□) of glucose. All experiments performed at 25°C, pH 7.0.

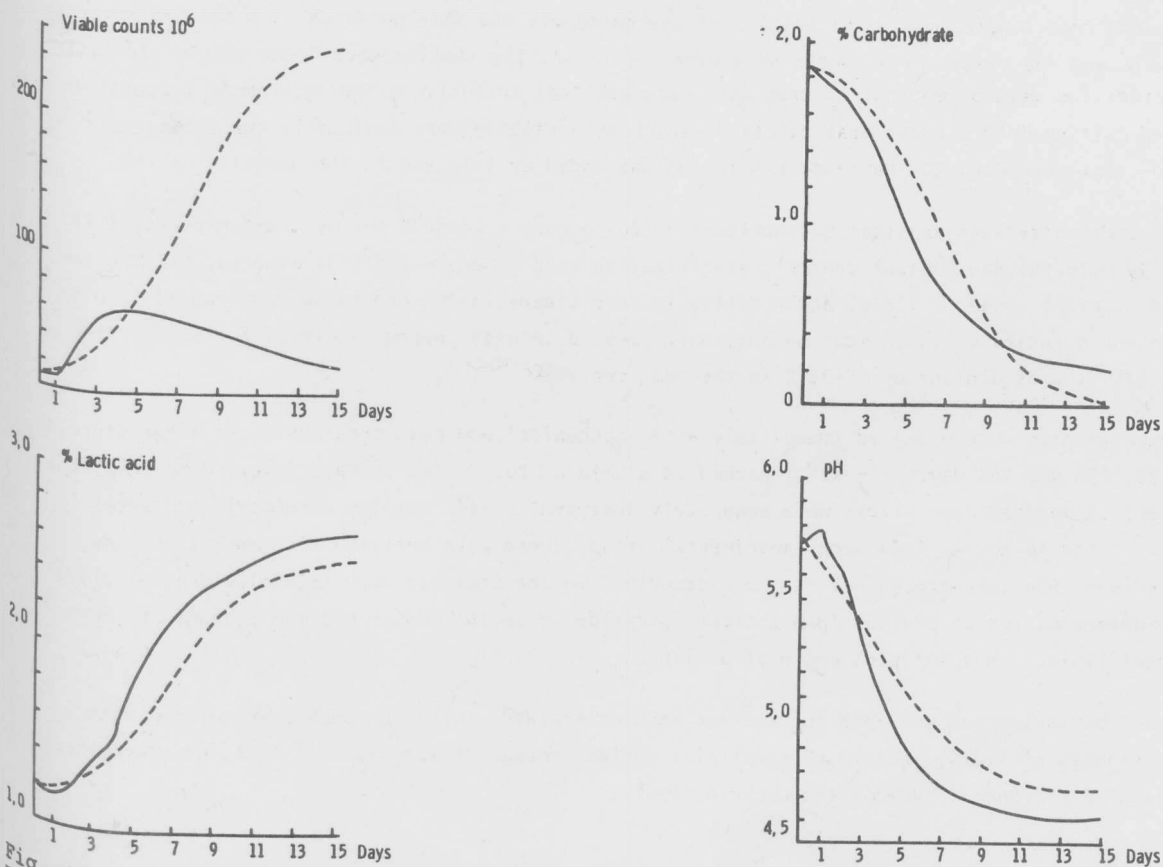


Fig. 4  
Relationship between measured and simulated (model) parameters during the fermentation process.  
Measured values (—) Simulated values (----)  
 $P_1 = 4 \cdot 10^{-2}$ ,  $P_2 = 2$ ,  $P_3 = 2 \cdot 10^{-3}$ ,  $P_4 = 2 \cdot 10^{-3}$ ,  $P_5 = 0,7$ ,  $P_6 = 1,37 \cdot 10^{-5}$ ,  $P_7 = -1,06$ .