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Development of mathematical model to describe the acidification occurring during the ripening of dry fermented sausage

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To describe the evolution of pH values during the ripening process of fermented sausages, the use of a mathematical model is proposed. The model is both easy to use and statistically adequate. It can be employed to distinguish the different stages in the acidification process associated with the fermentation of sausages. It can be successfully used in the control of the manufacture of sausages when this is carried out in temperature-controlled chambers equipped with a microprocessor to continuously record pH values.

The mathematical development of the function shows that the most rapid decline in pH occurs on the second day of ripening, coinciding with the highest levels of microbial development; on the 9th hour of the third day a stationary phase begins, which undergoes a slight increase from the 14th day.

In order to better interpret the process, various parameters involved in the proton concentration of the sausage mixture were determined: water activity, fermentative microbial population (*Lactobacillaceae, Micrococcaceae*) and metabolites derived from fermentative enzyme activity (lactic acid, acetic acid, total free fatty acids, total free amino acids).

INTRODUCTION

In the past, dry sausages have been manufactured using traditional craft methods and, despite increasing industrialization, the processes continues to be developed on an empirical basis. However, both commercial competitiveness and an increasing demand in the market for higher quality products has led to greater scientific attention being paid to the introduction of possible improvements in the conditions for ripening.

Over the last few decades the production of dry sausages has been optimized and standardized, particularly through the introduction of easily controlled fermentation chambers. Thus, it is now essential to use microprocessors or sensors that give a greater control over these industrial processes by continuously recording those parameters which are considered to be representative of the same. One such parameter which can be reliably monitored with sensors is pH (Rödel & Stiebing, 1989).

As a result of the activity of a fermentative microbial population, acidification (a decline of pH) occurs. The main metabolite responsible is lactic acid (Demeyer, 1982), although others are also present, such as acetic acid (Halvarson, 1972; Deketelaere *et al.*, 1974). Furthermore, the microbial lipolytic activity releases fatty acids which are a source of protons (Fournaud, 1976).

It has been shown that the acidification resulting from microbial activity on sugars, lipids and proteins determines the characteristics of sausages (Deketelaere *et al.*, 1974; Klement *et al.*, 1974; Astiasarán *et al.*, 1990). Hence it is important to control pH in order to guarantee the standardization and optimisation of the final product and the need to understand as much as possible about the development of acidification during the ripening process.

Demeyer *et al.* (1986) explained the kinetics of the metabolites formed during the fermentation of sausages using simple exponential equations. However, this type of function is unable to correctly describe pH development in all the aspects of an industrial process. To overcome this difficulty, it is necessary to apply more complex mathematical models such as those based on the Gompertz equation (Garthright, 1991), which have proved to be effective in describing the behaviour of micro-organisms in sausages (Bello & Sánchez-Fuertes, 1995).

To this end, a mathematical model capable of describing the evolution and principal characteristics of pH development has been applied to experimental pH values obtained during the ripening of a Spanish dry sausage.

MATERIALS AND METHODS

Preparation of the dry sausage

A dry fermented sausage was elaborated in a semi-industrial pilot plant using the formulation shown in Table 1 to which a starter culture of *Pediococcus pentosaceus* was added in quantity sufficient to give, in the ground meat, 10^7 c.f.u./g. The meat ingredients and the pork back fat were separately processed in a cutter and then put through a mincer fitted with the corresponding mincer plate. Once these ingredients had been mixed, they were transferred into a vacuum kneading machine, together with the remaining ingredients. The mixture was kneaded for 10 min under a vacuum to remove all air. Throughout the process the mixture was maintained at a temperature of 2°C. Finally, the mixture was stuffed into 70 mm diameter casings made of reconstituted collagen.

All the resulting sausages were transferred into a controlled chamber programmed according to the following values for temperature (°C), relative humidity (%), and time (h), thus giving seven successive stages:

Stage	Time (h)	Temperature (°C)	Relative humidity
1	24	20/22	85/95
2	24	20/22	75/85
3	24	18/20	73/83
4	24	16/18	71/81
5	24	14/16	69/79
6	120	12/14	67/77
7	until end	12/14	65/75

The ripening process was taken to be finished when the sausages were judged to have lost the necessary amount of humidity.

The elaboration process was repeated three times (A, B and C) under the same conditions so as to ensure uniformity of results. Four pieces from each batch were taken for analysis at the following times during the ripening process: 0, 3, 10, 17 and 24 days.

Methods

To prepare the samples for analysis, whole pieces were taken, their outer casings removed and they were then thoroughly cut up. Alternate pieces were chosen and mixed as much as was necessary to obtain a homogeneous and uniform mixture. All the steps were carried about at 2°C. PH was determined using the potentiometer Orion research microprocessor ionalyzer-901, with selective electrodes for solid samples (ISO, 1974; 2917–1974). Water activity was measured with an EEJA-3 Novasina apparatus. Lactic acid was determined using the enzymatic method of Noll (1974) with an enzymatic test from Boehringer Mannheim GmbH.

Acetic acid was measured by the method described by Duda *et al.* (1981) for the isolation and separation of short chain fatty acids. The dry sodium salts were dissolved in 3 ml of dichloromethane and the resulting solution was acidified with 0.01 ml of phosphoric acid. Quantitative determination was carried out using a Perkin–Elmer autosystem gas chromatograph with FID and Nukol capillary column (30 m 0.25 mm) following Ceccon *et al.* (1990). Oven temperature 172°C, detector temperature 220°C, injector temperature 220°C. Crotonic acid (C 4:1, w-2) was used as the internal standard.

Total free fatty acids were determined as an acidity value, using the international standard ISO, 1980; 1740–1980. Total free amino acids were determined by reaction with ninhydrin, according to the method of Massi (1963), which values them in mg of tyrosine per gram of dry matter. Microbial analyses were performed in the following manner: 10 g of sausage were homogenised into 90 ml peptone water (in sterile condition) during 2 min with a 'Stomacher'. Then, from this suspension, decimal dilutions in peptone water were prepared and spread on the corresponding plates. Plates were done with counts of De Man Rogosa and Sharpe agar (MRS, Oxoid) for lactic acid bacteria (30°C/72 h) in an anaerobic jar with a CO_2-enriched atmosphere (Gaspack, BBL) and with a staphylococcus medium N° 110 (Oxoid) for *micrococcaceae* analyses.

MATHEMATICAL AND STATISTICAL

Statistical analysis was carried out using statgraphics version 5.1 in its application for the analysis of variance. Similarly, where necessary, a multiple comparison Tukey t-test was carried out a *posteriori*. In order to describe and characterise pH development during the ripening of the dry sausage the mathematical model (Y) shown in Table 2 was applied. The meaning of each of the constants used in the mathematical function is also shown in the same table. To interpret the particular characteristics of the development of function Y, the maximum and minimum values given by the development of the first derivative Y' (rate of pH change) and the second derivative Y'' (acceleration in pH change) were determined.

Table 1. Formulation of Spanish dry sausage

Lean port (%)[a]	52
Pork trimmings (%)[b]	33
Pork back fat (%)[b]	15
Sugars (g/kg)	44
Curing salts (g/kg)[c]	24
Spanish paprika (g/kg)	20
Polyphosphates (g/kg)	2
Garlic (g/kg)	2

[a]Minced though a 22 mm plate.
[b]Minced though a 12 mm plate.
[c]Common salt with 130 p.p.m. nitrate and 130 p.p.m. of nitrite.

The values K_1 and K_4 are determined according to the experimental pH values found during the ripening process. The other constants (K_2, K_3, K_5, K_6) can be calculated with the help of the statgraphics programme, version 5.1, using its nonlinear regression application, based in the Marquart algorithm to determine the estimates that minimize the residual sum of squares.

RESULTS AND DISCUSSION

During the ripening of dry fermented sausages, acidification occurs which is reflected in the development of pH values throughout the ripening process. In our study, these values were measured in the samples taken at 0, 3, 10, 17 and 24 days of ripening in each of the batches (*A*, *B* and *C*) produced as is show in Table 3.

In order to check that the manufacturing process tested remained within normal parameters the development of the microbial load for fermentation was determined. Figure 1 shows the development of *Lactobacillus* sp., and *Micrococcaceae* as well as the variation in water activity as a determining parameter in the growth and inhibition of these micro-organisms.

When the proposed mathematical function (Table 2) is applied to the pH values from each batch, the values corresponding to the constants of the function are obtained, as can be seen in Table 4. For each batch the mathematical equation obtained presents a highly significant correlation coefficient (r^2: 0.900, $P < 0.001$). As the mathematical behaviour of each group is very similar, the subsequent study of the data on the evolution of acidification was made on the basis of the average values (Table 5) of 12 calculations (4 values ×3 batches). In this way, the following equation representing the development of pH is obtained:

$$\text{pH} = 5.85 - 1.04xe^{-e^{-0.92(D-2.29)}} + 0.13xe^{-e^{-0.45(D-16.2)}}$$

$$(r^2 = 0.997).$$

Figure 2 shows this function and the mathematical development of its first (Y') and second (Y'') derivatives in graph form. In the first case, a minimum value (v') is obtained representing the rate of pH change after 2 days and 6 h of ripening and a maximum value (V') after 16 days and 3 h. This minimum value marks the moment at which the rate of decline in pH is greatest and the maximum value marks the moment of greatest increase in pH seen towards the end of the ripening process.

In the second case, the graph of the development in the acceleration of pH change shows 3 minima (a''_1, a''_2, and a''_3) and two maxima (A''_1 and A''_2) which allows four stages in the acidification process (I; II; II and IV) to be clearly defined. The a''_1 minimum, occurring 30 h after the beginning of the ripening of the sausage, separates an initial stage (I) of latency in the release of protons

Table 2. Mathematical model proposed to study the evolution of pH during the ripening of dry fermented sausage and meaning of the elements which make up each function

$$Y = Y_0 - k_1^* e^{\left\{-e^{\left[-k_2^*(x-k_3)\right]}\right\}} + k_4^* e^{\left\{-e^{\left[-k_5^*(x-k_6)\right]}\right\}}$$

Y_0	Initial value of pH at the beginning of the ripening
X	Time of curing process (days)
k_1	Reduction of pH from an initial level to minimum value reached
k_2	Rate of reduction with relation to pH
k_3	Time at which the decline of pH is maximum
k_4	Increase of pH from the minimum value to the level at which the sausage experiences last day of ripening
k_5	Rate of relative increment to pH
k_6	Time in which the increment of velocity of pH is maximum

Table 3. Evolution of pH during the ripening of dry sausage in each of the three batches elaborated. Comparison by anova of one way

	A	B	C	Signification Level
0	5.83 ± 0.026	5.85 ± 0.015	5.87 ± 0.019	NS
3	5.23 ± 0.17	5.21 ± 0.17	5.26 ± 0.16	NS
10	4.80 ± 0.06	4.81 ± 0.013	4.83 ± 0.02	NS
17	4.87 ± 0.005	4.90 ± 0.020	4.86 ± 0.007	NS
24	4.93 ± 0.013	4.91 ± 0.012	4.94 ± 0.015	NS

NS $p > 0.05$.

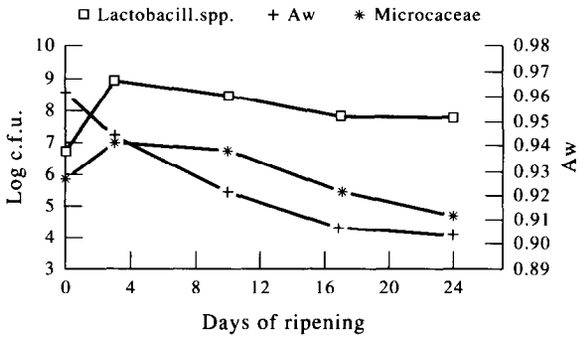

Fig. 1. Evolution of water activity and the fermenting micro-organisms during the ripening of dry sausage.

Table 4. Parameters which characterise the mathematical function and its mathematical development for the pH values in each experimental batch (A,B,C)

Parameter	A	B	C
Y_0	5.83	5.85	5.87
k_1	1.03	1.04	1.04
k_2	0.908	0.941	0.912
k_3	2.326	2.234	2.315
k_4	0.16	0.09	0.11
k_5	0.576	0.529	0.448
k_6	16.67	15.314	17.050
r^2	0.999	0.999	0.999

Table 5. Evolution of parameters which are involved in the acidification during the ripening of dry fermented sausage

Parameters[1]	Days of ripening				
	0	3	10	17	24
pH	5.85 ± 0.02^{a}	5.23 ± 0.02^{b}	4.81 ± 0.02^{c}	4.88 ± 0.01^{cd}	4.93 ± 0.01^{d}
Lactic acid (mmol/100g)	2.57 ± 0.04^{a}	4.22 ± 0.03^{b}	6.18 ± 0.04^{c}	6.13 ± 0.04^{c}	6.17 ± 0.02^{c}
Acetic acid (mmol/100 g)	0.58 ± 0.02^{a}	1.10 ± 0.02^{b}	2.68 ± 0.01^{c}	3.05 ± 0.04^{c}	3.06 ± 0.04^{c}
Total free fatty acid (mequiv/100 g)	3.18 ± 0.02^{a}	4.32 ± 0.03^{b}	6.33 ± 0.05^{c}	6.67 ± 0.05^{c}	6.72 ± 0.04^{c}
Total free aminoacids (mmol/tyrosine/100 g)	21.46 ± 0.85^{a}	26.53 ± 0.52^{b}	30.05 ± 0.57^{c}	36.42 ± 0.45^{d}	40.74 ± 0.72^{e}

Mean ($n = 12$) ± Standard error.
Means in the same line having different superscripts are significantly different ($p < 0.05$).

from a second stage (II) which is marked by an exponential decline in pH. During this period the metabolic activity of the fermentative micro-organisms gives rise to the formation of substances capable of releasing protons. The end of this stage is marked by the A''_1 maximum which occurs 3 days and 9 h into the ripening process.

This maximum marks the beginning of a third stage (III) in which the pH remains virtually constant, perhaps due to the existence of an acid-based equilibrium between the acidic substances and others which have formed that are alkaline. The second of the minimum values a''_2, occurring on day 11, coincides with that moment in the ripening process of the sausage when pH is lowest and can be interpreted as being the moment in which the release of protons has ceased.

The maximum A''_2, occurring at 14 days, is the starting point for a fourth stage (IV) in which pH increases slightly, perhaps due to the predominance of alkaline substances. The a''_3 minimum at 18 days and 9 h marks the moment in the ripening process as from which the marginal rise in pH becomes increasingly smaller.

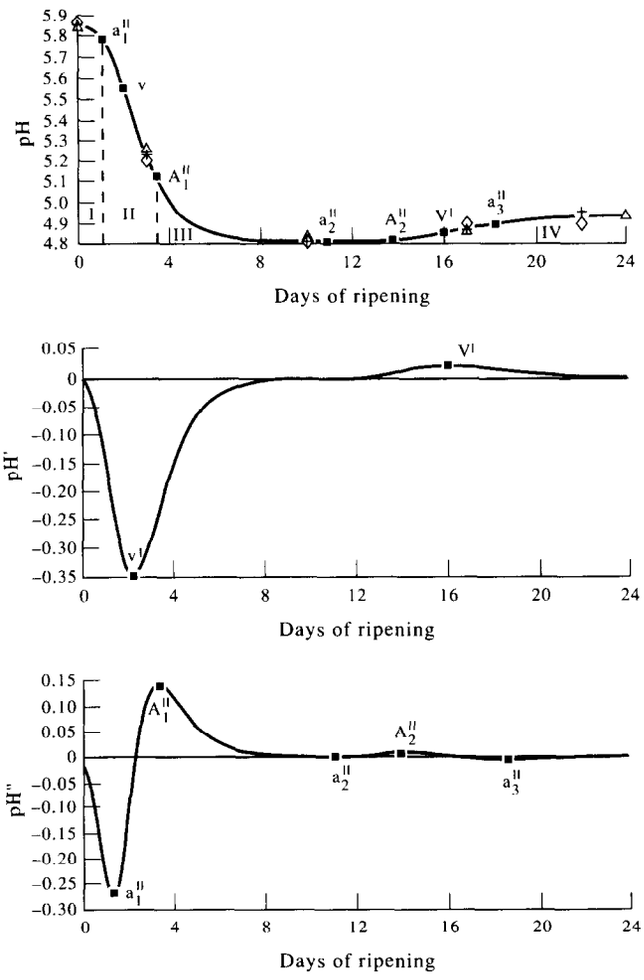

Fig. 2. Application of mathematical model to pH evolution during the ripening of dry fermented sausage (+, ▽, ◇ = average of each batch).

Indeed, an acidification process which must accurately reflect microbial development, and the effect of those metabolites produced, which are capable of modifying the proton concentration of the medium, is detected (Wardlaw *et al.*, 1973; Astiasarán *et al.*, 1990; Landvogt & Fischer, 1991).

Table 5 shows the values obtained for pH and the determination of the substances produced by microbial metabolism and which will determine the pH values of the sausage mixture: lactic and acetic acid, total free fatty acids, total free amino acids.

It is frequently acknowledged that all sugar metabolised by fermentative micro-organisms is transformed into lactic acid but some can also produce acetic acid (Liepe *et al.*, 1990). In our study lactic acid is predominant in the final product with a molar proportion of 2.0 in comparison with acetic acid. Although the maximum values for lactic (6.19 mM) and acetic (2.68 mM) acids were reached after 10 days of ripening and they remained constant until the end, it is worth pointing out that lactic acid formation was particularly more intense in the first three days.

Other acidic metabolites are the fatty acids released through the lipolytic activity of the micro-organisms. Valued on an acidity index, their development was parallel to that of lactic acid, as has been found for other types of sausage (Pyrcz & Pezacki, 1974).

During the fermentation of the sausage, an interaction occurs between the metabolism of carbohydrates and proteins which affects the proton concentration of the sausage mixture. At the initial stages of ripening, a proteolytic activity takes place, based on microbial endopeptidases (Vignolo *et al.*, 1988), which releases amino acids. Subsequently, proteolysis depends on meat cathepsins which are active throughout the ripening

process (Demeyer & Samejima, 1991; Demeyer, 1992). The micro-organisms themselves can metabolise the amino acids released from the proteins and convert them into more basic components which would account for the slight rise in pH seen at the end of the process.

In conclusion, the mathematical model proposed, when applied to the pH values obtained, provides a mathematical function which is both easy to use and statistically adequate, as is shown by its regression coefficient. The mathematical development of the first and second derivatives (rate of pH change and acceleration in the pH change, respectively) allows a complete description of the acidification process to be made, including the different stages which it comprises. Conclusions drawn from the model correspond to findings from chemical and microbiological analyses: the exponential decline in pH coincides with the development, which is also exponential in nature, of the fermentative micro-organisms and with the maximum production of acidifying metabolites. The stationary phases of the chemical and microbiological parameters also correspond to the stationary phase in pH variation.

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