MODELING OF MICROBIAL GROWTH IN REFRIGERATED DOUGHS FOR "EMPANADAS" WITH POTASSIUM SORBATE AND WHEY PROTEIN CONCENTRATE

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ABSTRACT

Dough for "empanadas", a traditional meal in Argentina, are sold refrigerated with the addition of preservatives. The replacement of a small percentage of flour by whey protein concentrate (WPC) improves the color and acceptability of the "empanadas", but can decrease the shelf-life due to the microorganisms incorporated with the WPC. In this work the effect of the addition of two selected concentrations of potassium sorbate (0.067 - 0.134%) to dough for "empanadas" prepared with and without WPC on yeast growth (predominant microorganisms in this product) during refrigeration storage was investigated. Gompertz equation and kinetic parameters as specific growth rate (μ), lag phase duration (LPD), maximum population density (MPD) and activation energies from an Arrhenius-type expression, were estimated. The addition of potassium sorbate increased the storage-life of the dough for "empanadas", prepared with and without WPC, three-four times. The effect of potassium sorbate was more important at the higher concentration assayed. Results allow to predict the growth of yeast in different samples of dough stored between 0 and 10C.

INTRODUCTION

The "empanadas", particularly those with meat filling, are a traditional meal in Argentina. The dough for "empanadas", with the addition of preservatives, is sold refrigerated in bakeries and supermarkets. Previous assays showed

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that the incorporation of a small percentage of whey protein concentrate (WPC) to the flour increases the extensibility and decreases the tenacity of dough, which is desirable in these products. Also, the addition of WPC improves the nutritional and sensory characteristics of the "empanadas". However, the addition of WPC could modify the microbial population of the dough, affecting its preservation and decreasing its shelf-life.

An aid in preventing fungal spoilage of mildly processed foods would be the application of the hurdle concept of the combined preservation method (Gould 1995). This method uses various hurdles which separately may not give adequate preservation but which, when combined, will give proper preservation. These hurdles may include lowering the temperature, water activity (by addition NaCl or sugar) or pH, or addition of preservatives. If the hurdles concept is to be applied successfully, the influence of various environmental factors on microbial growth need to be quantified.

Sorbic acid has known antimicrobial properties in a wide range of products. The salts of sorbic acid, especially the potassium salt, have important applications due to their high solubility in water. Early in its use, potassium sorbate was classified as relatively non-toxic. It was reported that sorbate can be metabolized by the organisms in a similar way to the naturally occurring fatty acids. The World Health Organization has stipulated for sorbate an Acceptable Daily Intake (ADI) of 25 mg/kg of body weight (Sofos and Busta 1981).

Mathematical models allow descriptions of microorganisms behavior and it is a helpful tool to improve food safety (Zwietering *et al.* 1990; Buchanan 1993). Generally, predictive models are built on the basis of data obtained from experiments run in liquid laboratory media. In these media different factors can be controlled more easily than in food products. The specific growth rate, and especially lag phase duration, can be quite different in a food compared to the values obtained in liquid media, even under the same environmental conditions. Thus, model validation in actual situations must be performed before the use of a model for predictive purposes (McMeekin *et al.* 1992). The goal of mathematical models, which allow calculation of lag phase and microbial growth rates, is to estimate microbial growth under storage conditions different from the originally tested ones (Buchanan 1993; Palumbo *et al.* 1991, 1992).

Low storage temperatures play a major role in extending product shelf-life but refrigeration temperatures are hardly kept constant during food handling. Temperature effects on microbial stability has been studied quantitatively by computer supported models based on heat transfer and microbial growth estimations (McMeekin and Olley 1986; Fu *et al.* 1991; Almonacid-Merino and Torres 1993; Li and Torres 1993). The effectiveness of chilling storage as a preservation method depends on the initial quality of the raw material and the time-temperature conditions. Commonly, during food transportation thermal oscillations reduce the product shelf-life.

The objective of the present work was to determine the effect of potassium sorbate (0.067 and 0.134%) and temperature (0; 4 and 10C) on the microbial growth in doughs for "empanadas" prepared with and without 3.2% WPC and to calculate the storage life. Microbial growth parameters were obtained by fitting adequate equations to microbial counts. This information would allow the prediction of microbial growth under conditions different from those tested experimentally but within the studied range of temperatures.

MATERIALS AND METHODS

Materials

Whey protein concentrate (WPC) was obtained by large scale ultrafiltration, and was a gift from Williner S.A. (Rafaela, Santa Fe, Argentina). WPC contained 5.1% moisture, 5.6% lipids, 5.95% ash, 49.3% protein (calculated as [total N (8.0%) - nonprotein N(0.3%)] × 6.38) and 32.3% lactose, estimated by difference.

Flour was type 000, and was a gift from Molino Campodónico (S.A. Miguel Campodónico Ltda, La Plata, Argentina). Flour contained 10.0% protein and 13.6% moisture.

Flour used in the preparation of doughs were: flour 000 (M1); flour 000 with 0.067% potassium sorbate (Anal-Item) (M2); flour 000 with 0.134% potassium sorbate (M3); flour 000 with 3.2% WPC (M4); flour 000 with 3.2% WPC plus 0.067% potassium sorbate (M5) and flour 000 with 3.2% WPC plus 0.134% potassium sorbate (M6). The concentrations of potassium sorbate were selected according to the values allowed by the Argentinean Regulations (Código Alimentario Argentino 1996) which establishes a maximum of 0.05% of sorbic acid in the dough.

Preparation of Dough for "Empanadas"

The dough for "empanadas" were prepared in a bakery under the usual conditions. The 400 g flour (with or without potassium sorbate or WPC), 160 g margarine (Dánica hojaldre), 2% salt (flour basis) and water, were mixed to obtain 800 g of cohesive dough. Dough was stretched, cut in dish shape and stored at 0, 4 and 10C.

pH and Water Activity (a_w) of Doughs

The pH was measured directly in the product using an insertion electrode (Ingold lot 405 m4, Urdof, Zurich, Switzerland). Water activity (a_w) was measured in a Novasina Thermoconstanter Humidat TH2/TH1 (Novasina, Zurich, Switzerland).

Microbial Growth

Microbial growth in dough for "empanadas" were analyzed at different storage times. Twenty grams of dough was homogenized with 80 mL 0.1% peptone in a stomacher during 1 min. Serial dilutions in 0.1% peptone water were made, and the appropriate dilution was plated on Plate Count Agar (PCA) for total microbial counts (48 h, 37C) and Yeast Glucose Cloranfenicol Agar (YGC) (Merck) for mold and yeast counts (5 days, 30C). Microbial counts were performed in duplicate. The isolated colonies were stained with Gram and methylene blue and observed through a light microscopy (Ortholux II, Leitz, Germany). Total and fecal coliform and *E. coli, Salmonella* spp and *Staphylococcus aureus* were tested by Methods 46016, 46117 and 46137, respectively, (AOAC 1984).

Modeling of Microbial Growth

The effect of the different formulations and temperature on microbial growth parameters was analyzed by using the modified Gompertz's equation (Gibson and Roberts 1989; Zwietering *et al.* 1990):

$$\log N = a + c \exp(-\exp(-b(t-m))) \qquad (1)$$

where: log N is the decimal logarithm of microbial counts [log (CFU/g)] at time t, a is asymptotic log count as time decrease indefinitely (approximately equivalent to log of the initial level of bacteria) [log(CFU/g)], c is log count increment as time increases indefinitely [log (CFU/g)], b is the maximum growth rate at time m [log (CFU/days)], m is time required to reach the maximum growth rate (days).

From these parameters, the following information were derived: specific growth rate μ = b.c/e [log (CFU/g) days^{-1}], with e = 2.7182; lag phase duration LPD = m - (1/b) [days], maximum population density MPD = a + c [log (CFU/g)].

Experimental Design and Statistical Analysis

Temperature, addition of WPC (0 and 3.2%) and potassium sorbate concentrations were examined by means of a full factorial design. Combinations between temperature (0; 4 and 10 $\pm$ 0.1C), with or without WPC (0 and 3.2%) and potassium sorbate (0; 0.067 and 0.134%) were assayed ($3 \times 2 \times 3 = 18$ experiments).

Data fits obtained from Gompertz model were analyzed by means of a statistical software (Systat 5.0, Evanston, Ill.). The Systat software calculates the set of parameters with the lowest residual sum of squares (RSS) and their 95%

confidence interval. Besides, it provides for each data fit, the sum of squares, the degree of freedom (DF) and the mean square due to the regression and the residual variation.

RESULTS AND DISCUSSION

Characterization of the Dough

The water activity (a_w) of samples varied between 0.999 and 0.986, and pH ranged between 6.5-6.6. These results indicate that doughs are good substrates for microbial growth. Microscopic observation showed that predominant microorganisms growing in different samples were yeasts. The study of microorganisms present in WPC showed 5.5 10^5 CFU/g total microbial counts, 1.8 10^2 CFU/g yeasts, 33 and 5.5 MPN/g total coliforms and fecal coliforms, respectively.

Fitting of Mathematical Model to Microbial Growth Curves

Fitting of Gompertz model to experimental data of microbial growth of yeasts in samples without WPC (M1, M2 and M3) and with WPC (M4, M5 and M6) at 0, 4 and 10C is shown in Fig. 1 a-f. At least 6 data points were obtained per curve and each point was the mean of two microbial counts. In all cases, a good agreement between experimental and predicted data was obtained.

The Gompertz parameters and the derived parameters (μ, LPD and MPD) for M1, M2, M3, M4, M5 and M6 samples stored at 0, 4 and 10C are shown in Table 1. Samples without potassium sorbate (M1 and M4) showed higher values of μ and, in samples stored at 0 and 4C, lower values of LPD, than samples containing potassium sorbate (Table 1). Moreover, samples with WPC (M4) stored at 0 and 4C, had higher μ values than control ones (M1), which suggests that WPC adds microorganisms that contribute to the deterioration of the product. Samples stored at 10C showed the highest values of μ and the lowest LPD values. On the other hand, potassium sorbate did not present an appreciable effect in samples stored at 10C (Table 1).

Inhibitory Effect of Sorbic Acid

The effect of sorbic acid on the growth parameters of molds and yeast was analyzed by considering that the antimicrobial action of weak acids is generally attributed to their undissociated fraction. Sorbic acid is a monoprotic weak acid with a dissociation constant Ka = 1.78 10^{-5} mol/L (pKa = 4.75). The undissociated concentration of a weak acid (uac) depends on the total concentration and pH, and was calculated as follows:

$$uac = Ct\ [H^+] \ /\ (Ka + [H^+]) \qquad (2)$$

where Ct is the total acid concentration and Ka is the dissociation constant of sorbic acid. The total concentrations of potassium sorbate used in the present work (Ct) were 11.5 mM and 23.0 mM (these values were calculated by considering that potassium sorbate was dissolved in the aqueous phase of dough (38.8 g of water), and pH of the samples was 6.55. By application of Eq. (2) the corresponding undissociated sorbic acid concentrations (uac) in mmol/L were 0.2 mM and 0.4 mM.

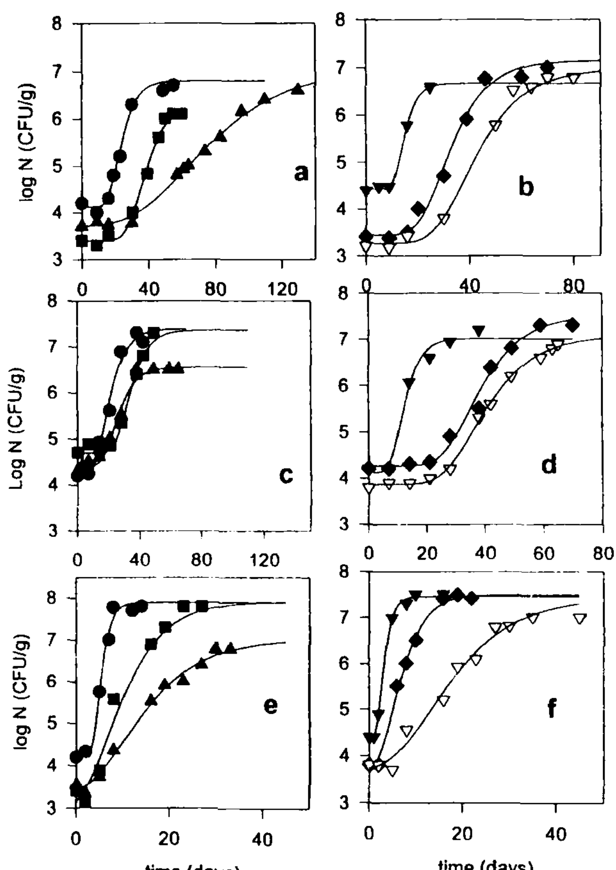

FIG. 1. FITTING OF THE GOMPERTZ MODEL TO YEAST COUNTS IN DOUGH STORED AT SELECTED TEMPERATURES: a, b: 0C; c, d: 4C; e, f: 10C

Flour used in the preparation of doughs: (•) M1: Flour 000; (■) M2: Flour 000 with 0.067% Potassium sorbate; (▲) M3: Flour 000 with 0.134% Potassium sorbate; (▼) M4: Flour 000 with 3.2% WPC; (◆) M5: Flour 000 with 3.2% WPC plus 0.067% potassium sorbate; (▽) M6: Flour 000 with 3.2% WPC plus 0.134% Potassium sorbate.

TABLE 1.
GOMPERTZ PARAMETERS (a, b, c and m) AND DERIVED PARAMETERS (μ, LPD AND MPD) OBTAINED BY APPLYING THE GOMPERTZ EQUATION TO THE GROWTH OF YEASTS IN DIFFERENT DOUGHS FOR "EMPANADAS" STORED AT DIFFERENT TEMPERATURES

M	T(°C)	a	b	c	m	μ	LPD	MPD
M1	0	4.13±0.09	0.16±0.03	2.68±0.27	21.52±0.27	0.16	15.45	6.81
M2	0	3.41±0.06	0.10±0.02	3.01±0.02	32.17±1.13	0.11	22.17	6.42
M3	0	3.71±0.05	0.03±0.01	3.38±0.35	62.88±1.90	0.04	29.54	7.09
M4	0	4.43±0.04	0.29±0.07	2.24±0.12	13.72±0.50	0.24	10.27	6.67
M5	0	3.45±0.13	0.10±0.02	3.70±0.24	29.44±1.44	0.14	19.73	7.15
M6	0	3.27±0.12	0.08±0.01	3.70±0.30	38.08±1.10	0.11	25.50	6.97
M1	4	4.22±0.18	0.14±0.05	3.17±0.36	17.90±1.66	0.20	10.49	7.39
M2	4	4.78±0.04	0.13±0.04	2.66±0.20	29..30±0.80	0.13	22.16	7.44
M3	4	4.43±0.04	0.13±0.02	2.23±0.14	32.15±1.20	0.11	24.45	6.66
M4	4	4.13±0.23	0.26±0.09	2.89±0.30	10.98±1.39	0.28	7.13	7.02
M5	4	4.08±0.13	0.17±0.03	2.82±0.30	22.16±0.80	0.18	16.30	6.98
M6	4	3.87±0.08	0.10±0.02	3.20±0.58	36.21±2.59	0.12	26.21	7.07
M1	10	4.26±0.17	0.70±0.29	3.63±0.40	4.23±0.30	0.93	2.80	7.89
M2	10	2.18±1.00	0.25±0.09	4.48±0.50	6.81±0.55	0.41	2.81	7.66
M3	10	3.24±0.30	0.13±0.03	3.78±0.56	10.59±1.52	0.16	2.90	7.02
M4	10	4.36±0.07	0.79±0.07	3.08±0.09	2.73±0.17	0.89	1.46	7.44
M5	10	3.68±0.18	0.28±0.04	3.81±0.37	5.38±1.06	0.40	1.80	7.49
M6	10	3.68±0.33	0.10±0.03	3.75±0.36	13.81±1.74	0.14	3.81	7.38

M: dough sample; a: asymptotic log count as time decrease indefinitely [log(CFU/g)], b: maximum growth rate at time m [log (CFU/days)]; c: log count increment as time increases indefinitely [log (CFU/g)]; m: time required to reach the maximum growth rate (days). μ: specific growth rate [log (CFU/g) d^{-1}]; LPD: lag phase duration [d]; MPD: maximum population density [log (CFU/g)]. Flour samples used in the preparation of doughs: M1: flour 000; M2: flour 000 with 0.067% potassium sorbate; M3: flour 000 with 0.134% potassium sorbate; M4: flour 000 with 3.2% WPC; M5: flour 000 with 3.2% WPC plus 0.067% potassium sorbate; M6: flour 000 with 3.2% WPC plus 0.134% potassium sorbate.

Figure 2 a-c shows the effect of undissociated acid concentration (uac) of potassium sorbate on the growth parameter μ, and Fig. 3 a-c shows the effect of uac on LPD, for samples prepared with or without WPC at different temperatures. It was observed that values of μ decreased when uac increased, mainly at 10C. Inversely, values of LPD increased when uac increased, in samples stored at 0 and 4C. The highest LPD values of samples stored at 0 and 4C were obtained in samples with the highest potassium sorbate content, which indicates the inhibitory effect of the preservative.

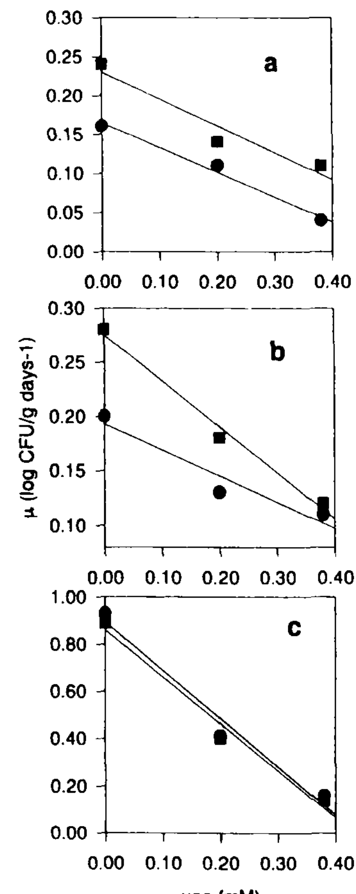

FIG. 2. VALUES OF SPECIFIC GROWTH RATE (μ) FOR YEAST IN DOUGH AS A FUNCTION OF UNDISSOCIATED SORBIC ACID CONCENTRATION (uac)
Storage temperature of dough: **a**: 0C, **b**: 4C, **c**: 10C. (•) Flours without WPC (M1, M2 and M3), (■) Flours with WPC (M4, M5 AND M6). M1, M2, M3, M4, M5 and M6 are the same as in Fig. 1.

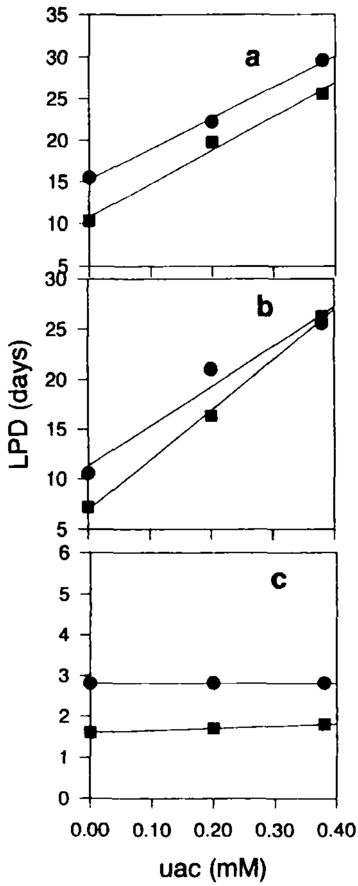

FIG. 3. VALUES OF LAG PHASE DURATION (LPD) FOR YEAST GROWN IN DOUGH AS A FUNCTION OF UNDISSOCIATED SORBIC ACID CONCENTRATION (uac)
Storage temperature of dough: **a**: 0C, **b**: 4C, **c**: 10C. (•) Flours without WPC (M1, M2 and M3), (■) Flours with WPC (M4, M5 and M6). M1, M2, M3, M4, M5 and M6 are the same as in Fig. 1.

Storage Temperature and Microbial Growth Parameters

The effect of storage temperature (0, 4 and 10C) on different parameters of microbial growth was analyzed.

Exponential Growth Rate: The effect of storage temperature on the specific growth rate (μ) for the microorganisms growing in M1, M2, M3, M4, M5 and M6 samples was determined using different models.

Arrhenius Model

$$\mu = A \exp(-E_\mu/RT) \tag{3}$$

where μ is the specific growth rate [log(CFU/g) days^{-1}], T the absolute temperature [K], E_μ is the activation energy [KJoule/mol], A is the preexponential factor [log(CFU/g)days^{-1}] and R the gas constant [8.31 Joule/K mol].

Linear Model

$$\mu = \mu_0 + d\,T \tag{4}$$

where μ is the specific growth rate [log(CFU/g) days^{-1}] evaluated at T (C), μ_0 is the value of μ at 0C [log(CFU/g) d^{-1}], and d is the slope of the linear regression [log (CFU/g) d^{-1} C^{-1}] (Li and Torres 1993; Spencer and Baines 1964).

Square Root Equation. Ratkowsky *et al.* (1983) proposed the following relationship:

$$\sqrt{\mu} = g\,(T'-T_0') \tag{5}$$

where g is a regression coefficient [log CFU/g days^{-1}]$^{1/2}$ K^{-1}, T′ is the incubation temperature [C], T_0' is a conceptual temperature, with no metabolic meaning for psychrophiles, psychrotrophs and mesophiles (Ratkovwsky *et al.* 1983). Equation (5) was modified as follows:

$$\sqrt{\mu} = p + q\,T \tag{6}$$

where p is derived by extrapolating the linear regression to zero [log CFU/g d^{-1}]$^{1/2}$, q is the slope of the regression line [log (CFU/g) d^{-1}]$^{1/2}$ C^{-1}, and T is the temperature [C].

The parameters obtained for the three models are listed in Table 2. The correlation coefficients (R^2) were compared in order to select a model for the temperature dependence, having the Arrhenius model the highest correlation. In this model, the activation energy of a microorganism can be seen as a measure of the sensitivity of its growth rate to temperature changes. Figure 4 a and b shows the fit of the Arrhenius equation to the yeast growth in samples treated with different concentrations of potassium sorbate, with and without WPC, respectively. The highest value of E_μ (116.88 KJoule/mol) was obtained in samples without the addition of potassium sorbate and without WPC (M1). Samples with potassium sorbate and WPC (M5 and M6) presented the lowest E_μ values (68.6 and 38.6 KJoule/mol, respectively).

TABLE 2.
APPLICATION OF ARRHENIUS, LINEAR AND SQUARE ROOT MODELS TO EVALUATE TEMPERATURE AND THE SPECIFIC GROWTH RATE OF YEAST GROWING IN SELECTED DOUGHS

	Arrhenius model			Linear model			Square root model		
Sample	LnA	E_μ	R^2	μ_o	d	R^2	q	p	R^2
M1	49.5	116.8	0.92	0.05	0.08	0.87	0.50	0.0	0.99
M2	36.1	83.4	0.92	0.07	0.03	0.88	0.35	0.02	0.89
M3	36.5	90.0	0.96	0.04	0.02	0.99	0.006	0.02	0.96
M4	36.8	87.1	0.92	0.13	0.07	0.88	0.03	0.08	0.88
M5	28.2	68.6	0.97	0.12	0.02	0.93	0.01	0.06	0.93
M6	4.6	38.2	0.99	0.11	0.003	0.94	0.001	0.05	0.94

A: log(CFU/g)d^{-1}; E_μ: Kjoule/mol; μ_o: log(CFU/g) d^{-1}; d: log(CFU/g)d^{-1} C^{-1} ; q: (logCFU/g $d^{-1})^{1/2}$ C^{-1}, p: (logCFU/g)$d^{-1})^{1/2}$. Flours used in the preparation of doughs: M1: flour 000; M2: flour 000 with 0.067% potassium sorbate; M3: flour 000 with 0.134% potassium sorbate; M4: flour 000 with 3.2% WPC; M5: flour 000 with 3.2% WPC plus 0.067% potassium sorbate; M6: flour 000 with 3.2% WPC plus 0.134% potassium sorbate.

Lag Phase Duration (LPD). When considering product shelf-life, the lag phase duration (LPD) of damage-causing microorganisms is an important parameter. Zwietering *et al.* (1991) modified the extended Ratkowsky model to describe the lag time as a function of temperature. The effect of temperature on LPD reflects how the adaptation period of microorganisms to their new environment changes with the temperature. In this regard, the adaptation rate is the reciprocal of LPD (Li 1988; Li and Torres 1993), and was fitted to an Arrhenius-type model:

$$1/LPD = D \exp(-E_{1/LPD}/RT) \quad (7)$$

where 1/LPD is the adaptation rate [$days^{-1}$] at T [K], D is the preexponential factor [d^{-1}], E_{LPD} is the activation energy [KJoule/mol], and R is the gas constant [8.31 Joule/K mol]. Figure 5a and b shows the linear fit of the adaptation rate of yeast in dough with and without WPC, as a function of temperature. A linear relationship was obtained for all cases studied, with correlation coefficients (R^2) ranging from 0.85 to 0.99 (Table 3). Yeast growing in M1 and M5 had lower E_{LPD} values (97.80 and 96.15 KJoule/mol, respectively). Yeast growing in samples with potassium sorbate and without WPC (M2 and M3) was the most sensitive, with E_{LPD} values of 156.60 - 197.03 Kjoule/mol.

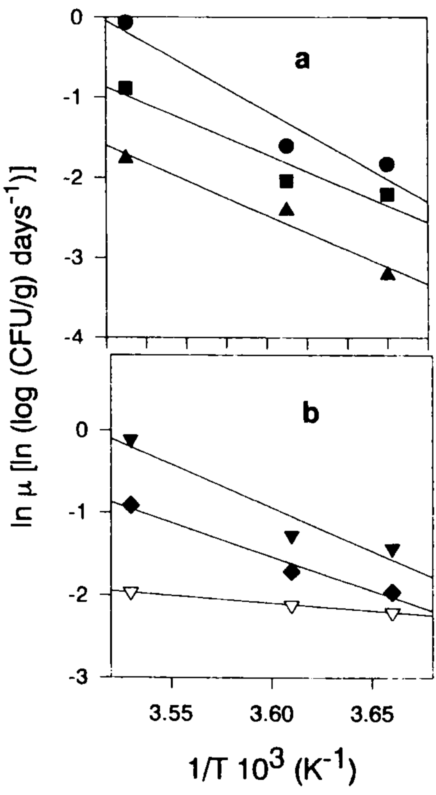

FIG. 4. APPLICATION OF ARRHENIUS MODEL TO EVALUATE THE TEMPERATURE DEPENDENCE OF THE EXPONENTIAL GROWTH RATE FOR YEAST IN DOUGHS
(a): Flours without WPC (•) M1, (■) M2, (▲) M3, (b): Flours with WPC (▼) M4, (◆) M5 and (▽) M6. M1, M2, M3, M4, M5 and M6 are the same as in Fig. 1.

Correlation of Lag Phase Duration (LPD) and the Reciprocal of Exponential Growth Rate (μ)

An early report (Cooper 1963) noted that in some examples the ratio of growth rate to generation time was nearly constant. This suggested a linear relationship between lag time and the reciprocal of exponential growth rate, which was confirmed in the present work for yeast growing in different dough samples. A linear relationship between $1/\mu$ and LPD was proposed for all the cases, and the correlation coefficients obtained ranged from 0.69 and 0.98. Figure 6a, b and c shows the correlation obtained for yeast in dough with and without WPC. Li and Torres (1993) also found a linear relationship for *P. fluorescens* growing in a medium with NaCl or glycerol as the a_w controlling solute.

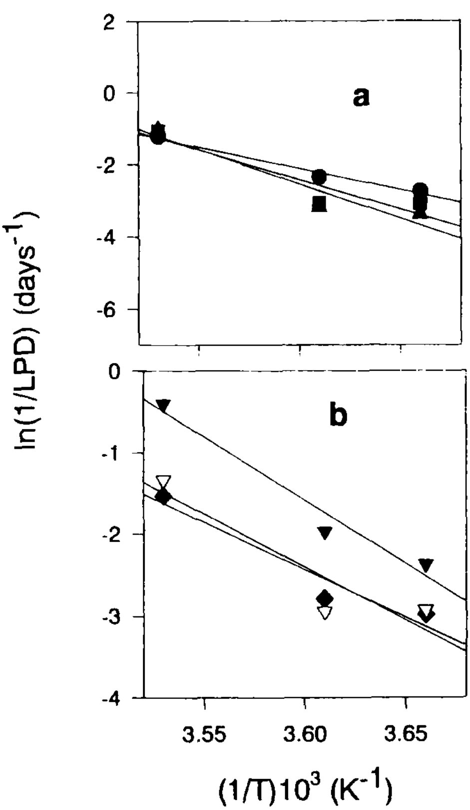

FIG. 5. ARRHENIUS PLOT OF THE ADAPTATION RATE (1/LPD) FOR YEAST IN DOUGHS

(a): Flours without WPC (•) M1, (■) M2, (▲) M3, (b): Flours with WPC (▼) M4, (◆) M5 and (▽) M6. M1, M2, M3, M4, M5 and M6 are the same as in Fig. 1.

TABLE 3.
APPLICATION OF THE ARRHENIUS MODELS TO EVALUATE THE TEMPERATURE EFFECT ON ADAPTATION RATE (1/LPD) OF YEAST GROWING IN SELECTED DOUGHS

Samples	ln D	$E_{1/LPD}$	R^2
M1	40.28	97.8	0.98
M2	65.96	156.6	0.99
M3	83.05	197.0	0.98
M4	54.38	128.8	0.96
M5	39.24	96.2	0.96
M6	44.28	107.7	0.85

D: d^{-1}; $E_{1/LPD}$: Kjoule/mol. Flours used in the preparation of doughs: M1: flour 000; M2: flour 000 with 0.067% potassium sorbate; M3: flour 000 with 0.134% potassium sorbate; M4: flour 000 with 3.2% WPC; M5: flour 000 with 3.2% WPC plus 0.067% potassium sorbate; M6: flour 000 with 3.2% WPC plus 0.134% potassium sorbate.

The present work allowed to predict the microbial growth under different temperature conditions by means of the activation energy values for LPD and μ, derived from an Arrhenius type model. On the basis of microbial testing in foods is expensive and time consuming, mathematical models become a useful tool to provide a matrix of microbial growth responses to a broad range of storage conditions often observed in the distribution chain of the product.

Storage Life

The storage life was defined as the time needed to achieve concentrations of yeast of $10^{6\pm0.2}$ CFU/g and absence of pathogen microorganisms (Bracket 1994; Giannuzzi 1998). Confidence limits of the storage life were calculated by considering the average experimental error in the microbial counts (0.2 log CFU/g). Neither *E. coli* nor *Salmonella* were found in the systems studied in the

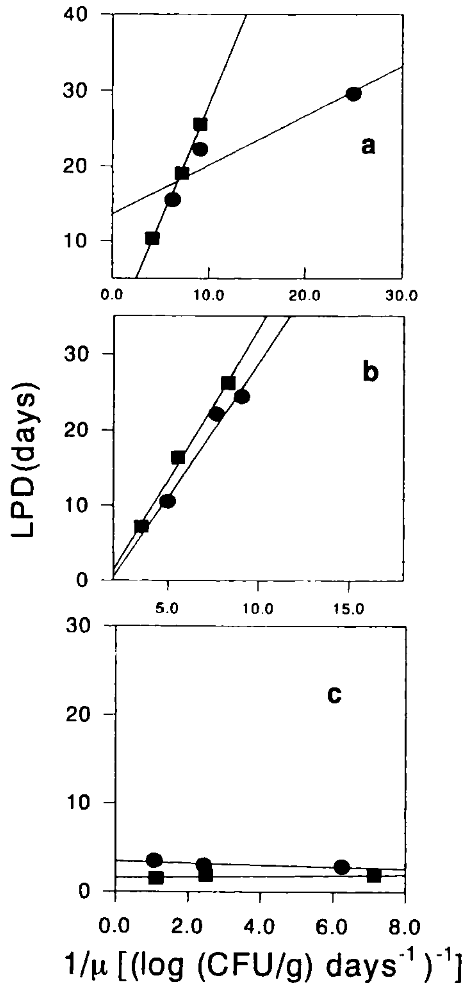

FIG. 6. CORRELATION OF LAG PHASE DURATION (LPD) AND THE RECIPROCAL OF SPECIFIC GROWTH RATE ($1/\mu$) FOR YEAST IN DOUGHS

Storage temperature of dough: (**a**): 0C, (**b**): 4C and (**c**): 10C. (•) Flours without WPC (M1, M2 and M3), (■) Flours with WPC (M4, M5 and M6). M1, M2, M3, M4, M5 and M6 are the same as in Fig. 1.

TABLE 4.
STORAGE LIFE (TIME TO REACH COUNTS $10^{6\pm0.2}$ CFU/G) OF DOUGHS FOR "EMPANADAS" STORED AT DIFFERENT TEMPERATURES

Dough	Storage life (days)		
sample	0C	4C	10C
M1	28 ± 2.0	22 ± 2.0	5.4 ± 0.3
M2	51 ± 7.1	31 ± 2.0	14.4 ± 1.6
M3	101 ± 8.0	40 ± 3.0	19 ± 2.0
M4	18 ± 2.2	14 ± 1.2	3.4 ± 0.2
M5	40 ± 2.0	28 ± 2.1	8 ± 0.6
M6	53 ± 6.0	45.5 ± 3.0	19 ± 1.5

Flours used in the preparation of doughs: M1: flour 000; M2: flour 000 with 0.067% potassium sorbate; M3: flour 000 with 0.134% potassium sorbate; M4: flour 000 with 3.2% WPC; M5: flour 000 with 3.2% WPC plus 0.067% potassium sorbate; M6: flour 000 with 3.2% WPC plus 0.134% potassium sorbate.

present work (M1, M2, M3, M4, M5, M6), and the concentration of *S. aureus* was lower than 10^2 UFC/g. Table 4 shows the storage life obtained for the different dough (M1, M2, M3, M4, M5 and M6) at the three temperatures studied. The addition of potassium sorbate increased the storage life of doughs, with respect to control condition (M1 and M4) at the three temperatures studied, being more evident at 0C. The effect of potassium sorbate was more important at the higher concentration assayed. The addition of WPC (M4) decreased the storage life of 10, 8 and 2 days with respect to M1 at 0, 4 and 10C, probably due to the microorganisms incorporated with the whey. The addition of potassium sorbate to the samples containing WPC (M5 and M6) increased the enhancement of the nutritional and sensorial properties of dough due to WPC with a higher storage life.

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