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Modelling of Microbial Growth in Potato Homogenate

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Abstract: The use of linear and non-linear models (Gompertz and logistic equations) to fit changes in microbial counts in a model system of potato homogenate at various concentrations of chemical preservatives (citric and ascorbic acids) was evaluated. The effect of undissociated acid concentrations (UAC) on μ (specific growth rate), lag phase duration and inactivation rate of Enterobacteriaceae, *Lactobacillus* sp, *Pseudomonas* sp and psychrotrophic microorganisms was determined. Citric acid had a strong inhibitory action on growth rate at low concentrations (0.065 mM UAC, pH = 5). *Pseudomonas* sp were the microorganisms most inhibited by citric acid. Ascorbic acid, at low UAC concentrations (0.302 mM UAC) was more inhibitory to Enterobacteriaceae than to the other microorganisms. For UAC concentration lower than 3 mM, μ values of all the microorganisms tested were higher with ascorbic acid than with citric acid; however at higher concentrations (>10 mM) both acids had similar effects on μ.

Key words: mathematical models, microbial growth, preservatives, citric and ascorbic acid, potato homogenate.

INTRODUCTION

Microbial spoilage of food is of great concern to producers, retailers and consumers. Growth of either pathogenic or spoilage microorganisms is unwanted with regard to food safety as well as from an economic point of view.

In describing the behaviour of microorganisms under different conditions, the use of mathematical models is receiving great attention as they allow the prediction of microbial safety or shelf-life of products. Probabilistic models are appropriate where the concern is toxin production, while kinetic models are suitable for spoilage or food-poisoning microorganisms (Buchanan 1993). Kinetic models provide quantitative information about lag time and microbial growth rate during refrigerated storage. Predictive models allow the evaluation of the effects of factors like pH and preservatives under aerobic and anaerobic conditions on the growth of pathogenic microorganisms, especially when associated with low temperatures (Palumbo 1991, 1992).

Pre-peeled potatoes are perishable products which can be protected from both enzymatic action and microbial growth by the addition of chemical preservatives. Preservatives like ascorbic and citric acid constitute attractive alternatives because these acids appear naturally in many foods, show an antimicrobial effect and are generally recognised as safe.

In order to determine the effects of these preservatives, a potato homogenate was used because applied concentrations could be more easily controlled than is possible in whole food products.

The objectives of the present work were as follows:

(1) To evaluate the feasibility of using linear and non-linear models to fit changes in microbial counts in a model system of potato homogenate to which defined concentrations of chemical preservatives (citric and ascorbic acids) were added.
(2) To determine the effect of undissociated acid concentrations on the representative parameters

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of microbial growth or inhibition, using appropriate indices to quantify these effects.

MATERIALS AND METHODS

Potato samples (*Solanum tuberosum* (L), Kennebec variety from Balcarce, Argentina), stored for 2 months at 6°C, were washed and hand-peeled.

The model system consisted of 10 g potato homogenate obtained by blending peeled potatoes without addition of liquid. The samples were placed in test tubes and chemical preservatives (citric and ascorbic acids) were added in known concentrations; this allowed us to determine the inhibitory effect of each preservative. The water content of potato tissue was taken into account in calculating the concentration of added preservatives. After addition of the preservative, a Vortex Type 37 600 Mixer (Barnsted, Thermolyne) (50% sample volume, 50% head volume) was used to obtain a uniform distribution. The control samples consisted of the same homogenates without acid addition (pH = 5·9–6·1). Citric acid was added at the following concentrations: 12·90, 16·80, 24·03, 48·07 and 72·11 mM; the corresponding homogenate pH values were 5·1, 5·0, 4·5, 4·3 and 3·7, respectively. Ascorbic acid concentrations were 10·40, 15·60, 26·04, 52·08 and 78·12 mM and the corresponding pH values were 6·0, 5·8, 5·0, 4·5 and 4·4, respectively. Test tubes were packed in EVA/SARAN/EVA film (Grace, Quilmes, Argentina), being EVA ethyl vinyl acetate and SARAN polyvinyl and polyvinylidene chloride copolymer (water vapour permeability, WVP = 7·2 g m^{-2} atm^{-1} day^{-1} at 30°C and RH = 78%, oxygen transmission rate, OTR = 37 cm^3 m^{-2} atm^{-1} day^{-1}) with partial gaseous evacuation in a Minidual equipment model MW 4980 (Scholnik SAIC, Argentina). Manometric pressure in the vacuum chamber was 4·5 mmHg; samples were stored at 4°C.

Microbiological analysis

The composition of the microflora in untreated potatoes at the beginning and end of the storage period, was determined by analysing the colonies that grew in selective media and was reported in a previous study (Giannuzzi and Zaritzky 1993).

During storage the following analyses were performed in triplicate:

(a) Psychrotrophic microorganisms: 1 ml of each dilution was inoculated into Plate Count Agar (Merck, Darmstadt, Germany) and incubated at 4°C for 7 days.

(b) Enterobacteriaceae counts: 0·1 ml of each dilution was inoculated into Caso agar (casein–peptone soymeal–peptone broth) (Merck) overlayed with red bile violet dextrose agar (Merck), which had been melted and cooled to 45°C. The plates were incubated at 37°C for 18–24 h.

(c) *Pseudomonas* sp: There were determined by inoculating 0.1 ml of each dilution into Masurovsky agar (Masurovsky 1963) with incubation at 30°C for 2 days.

(d) *Lactobacillus* sp: 0.1 ml of each dilution was inoculated into Caso agar (Merck) overlayed with Rogosa agar (*Lactobacillus* selective agar) (Merck) which had been melted and cooled to 45°C, with incubation at 30°C for 3 days in anaerobic conditions.

Modelling of microbial growth

Mathematical models allowed us to analyse the effect of different chemical preservatives on microbial growth parameters. One of the recommended models (Zwietering *et al.* 1990) is the Gompertz growth equation whose expression is

$$\log N = \log N_0 + a.\exp(-\exp(-b.(t-m))) \quad (1)$$

where log N is the decimal logarithm of microbial count (log(CFU g^{-1})) at time t; log N_0 is the asymptotic log count as time decreases indefinitely (approximately equivalent to the log of the initial level of bacteria) (log(CFU g^{-1})); a is the count increment as time increases indefinitely, that is number of log cycles of growth (log(CFU g^{-1})); m is the time required to reach the maximum growth rate (days); and b is the specific growth rate at time m (day^{-1}).

From these parameters, the maximum specific growth rate ($\mu = b.a/e$ (log(CFU g^{-1}) day^{-1}), where $e = 2{\cdot}7182$) and the lag phase duration (LPD = $m - (1/b)$ (days)), were derived.

A logistic model (symmetrical curve) was also applied in order to test its suitability:

$$\log N = \log N_0 + a/(1 + \exp(d - c.t)) \quad (2)$$

where log N and log N_0 have the same meanings as above; d is a dimensionless parameter; and c is the specific growth rate at the half-time value of the exponential phase (day^{-1}).

From these parameters, the exponential microbial growth rate ($\mu = a.c/4$ (log(CFU g^{-1}) day^{-1}), and the lag phase duration (LPD = $(d - 2/c)$(day)), were derived.

The equations were fitted to the growth data by non-linear regression using Systat software (Systat, Evanston, IL, USA). The selected algorithm calculates the set of parameters with the lowest residual sum of squares (RSS) and their 95% confidence interval for the different bacteria tested. Data were collected at various concentrations of citric and ascorbic acids. Modelling was applied to every culture in which microbial growth was

detected. In those cases where microorganisms did not show sigmoid curves, straight lines were fitted to the data for log CFU g^{-1} *vs.* time.

$$\log N = \log N_0 + \mu(t - \text{LPD}) \quad (3)$$

When the preservatives showed a bacteriostatic effect, exponential growth rates (μ) were close to zero. When a bactericidal effect was observed, μ was replaced by the inactivation rate (IR) which has negative values. LPD was defined as the storage time for which changes in microbial counts with respect to initial values remained lower than 0·5 log units of CFU g^{-1}.

The exponential growth rate (μ) and the inactivation rate (IR) were obtained from the slopes of the linear regressions.

Statistical analysis

In order to compare the results obtained by the application of the different mathematical models and to study the effects of preservative concentration data fits obtained by the application of the Gompertz and logistic models were compared statistically using the F ratio test.

The Systat software provides, for each data fit, the residual sum of squares (RSS). Even for non-linear models it can be considered that the variance ratio is approximately F-distributed when the sample size is large (Zwietering 1990). Experimental F values were calculated by dividing the larger variance by the smaller one. Degrees of freedom (number of datum points − number of model parameters) were equal for the two models because they have the same number of parameters. These degrees of freedom corresponding to both variances (in numerator and denominator) were used to obtained the F table.

RESULTS AND DISCUSSION

Considering that the antimicrobial action of a weak acid is generally attributed to the undissociated fraction, its effect on microbial growth parameters was analysed for each microorganism.

Undissociated fraction of the weak acids

The undissociated concentrations of the weak acids (UAC) can be calculated by the following equations:

For a diprotic acid (ascorbic acid)

$$[AH_2] = \frac{C_a[H^+]^2}{[H^+]^2 + [H^+]K_1 + K_1K_2} \quad (4)$$

For a triprotic acid (citric acid)

$$[AH_3] = \frac{C_a[H^+]^3}{[H^+]^3 + [H^+]^2K_1 + [H^+]K_1K_2 + K_1K_2K_3} \quad (5)$$

where $[AH_2]$, $[AH_3]$ are the undissociated acid concentrations for the diprotic and triprotic acids respectively, C_a is the total acid concentration, K_1, K_2, K_3 are the dissociation constants of the acids.

Equations (**4**) and (**5**) were applied to calculate the concentrations of undissociated ascorbic and citric acids at various values of pH, giving values of pK_1, pK_2, pK_3 for citric acid of 3·14, 4·77 and 6·69, respectively; for ascorbic acid pK_1 and pK_2 were 4·0 and 11·79, respectively. The values of undissociated acid concentration (UAC) of ascorbic and citric acids are shown in Table 1. It can be observed that at the same pH value (eg pH = 5), the undissociated concentration of ascorbic acid is 45 times that of citric acid.

The Gompertz and logistic equations were fitted to microbial counts of *Lactobacillus* sp, Enterobacteriaceae, *Pseudomonas* sp and psychrotrophic microorganisms which were collected at different concentrations of citric and ascorbic acids; original data were reported in previous work (Giannuzzi and Zaritzky 1993). The Gompertz and logistic models were applied to every culture in which microbial growth was detected and they allowed the prediction of the entire growth curve. In all cases under study, good agreement between experimental data and predicted values was obtained. A minimum of 12 points was taken for each growth curve. Linear regressions were fitted when bacteriostatic or bactericidal effects were observed. Some examples of the sigmoidal curves and straight line fitting are shown in Fig 1 for *Lactobacillus* sp in potato homogenate treated with different concentrations of ascorbic acid. Regression coefficients (R^2 = 1 − residual/total) were in all cases higher than 0·990 for both models tested. Non-significant differences were observed ($P < 0·05$) between the two models when they were compared in their fitness using the F test. The experimental F values for comparison of the models

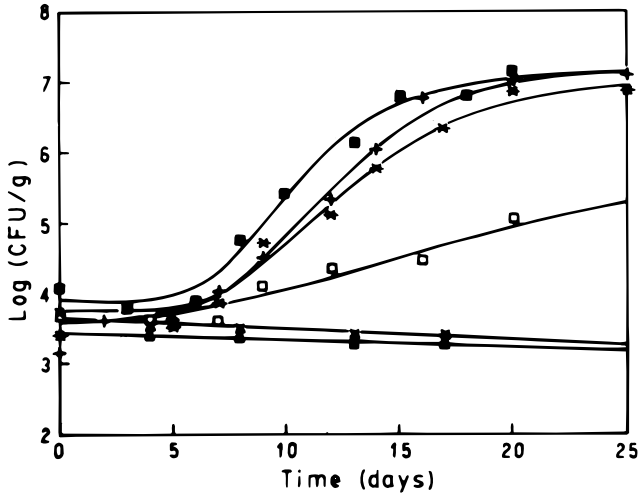

Fig 1. Examples of the sigmoidal curves and straight line fitting for *Lactobacillus* sp in potato homogenate treated with ascorbic acid: ■, Control; +, 0·125 mM, ★, 0·302 mM; □, 2·910 mM; ×, 14·80 mM; ▲, 25·50 mM.

TABLE 1
Gompertz, logistic and derived parameters for the studied microorganisms at different concentrations of critic and ascorbic acids

	pH	*Total concentration* (*m*M)	*UAC* (*m*M)	*Gompertz* (*eqn* **1**)				*Logistic* (*eqn* **2**)				*Derived parameters*[a]	
				log N_0	*a*	*b*	*m*	*log* N_0	*a*	*d*	*c*	μ	*LPD*
Lactobacillus sp	6·0	Control	0	3·910	3·196	0·334	9·18	3·854	3·150	5·169	0·502	0·393	6·193
Ascorbic acid	6·0	10·40	0·125	3·605	3·644	0·268	10·41	3·548	3·584	4·981	0·421	0·359	6·685
	5·8	15·60	0·302	3·649	3·416	0·233	10·65	3·581	3·354	4·372	0·359	0·293	6·356
	5·0	26·04	2·910	3·784	1·732	0·164	13·96	3·760	1·758	4·342	0·263	0·104	7·867
Citric acid	5·1	12·90	0·023	3·540	1·591	0·628	12·56	3·538	1·561	13·872	1·052	0·423	11·135
	5·0	16·60	0·065	3·493	0·632	1·197	13·73	3·493	0·633	26·026	1·828	0·278	12·900
Enterobacteriaceae	6·0	Control	0	4·515	1·839	0·346	7·78	4·481	1·833	4·653	0·525	0·234	4·895
Ascorbic acid	6·0	10·40	0·125	4·307	1·333	0·463	10·72	4·306	1·287	9·449	0·819	0·227	8·566
	5·8	15·60	0·302	4·186	1·169	0·338	16·53	4·188	0·937	12·976	0·768	0·145	13·575
Citric acid	5·1	12·90	0·023	4·648	1·412	0·398	12·67	4·588	1·414	9·025	0·679	0·207	10·164
	5·0	16·60	0·065	4·400	1·089	0·304	14·72	4·375	1·084	8·710	0·533	0·122	11·435
Pseudomonas sp	6·0	Control	0	4·697	2·541	0·929	6·23	4·693	2·533	8·579	1·276	0·869	5·156
Ascorbic acid	6·0	10·40	0·125	4·512	2·197	1·005	5·91	4·509	2·189	10·135	1·599	0·812	4·920
	5·8	15·60	0·302	4·420	1·621	1·417	5·60	4·420	1·619	12·685	2·088	0·845	4·895
	5·0	26·04	2·910	4·578	1·057	0·287	10·84	4·577	0·960	7·009	0·591	0·112	7·356
Citric acid	5·1	12·90	0·023	4·593	1·924	0·349	9·44	4·637	2·150	4·844	0·383	0·247	6·576
	5·0	16·60	0·065	4·676	1·058	0·484	10·9	4·686	1·029	10·189	0·832	0·188	8·928
Psychrotrophic microorganisms	6·0	Control	0	4·274	2·842	0·670	5·34	4·171	2·933	5·162	0·870	0·700	3·855
Ascorbic acid	6·0	10·40	0·125	4·248	2·811	0·637	5·55	4·212	2·820	5·386	0·870	0·659	3·988
	5·8	15·60	0·302	4·227	2·404	0·697	5·32	4·193	2·431	5·519	0·941	0·616	3·890
	5·0	26·04	2·910	4·251	1·851	0·252	8·04	4·185	1·866	3·496	0·373	0·172	4·076
Citric acid	5·1	12·90	0·023	4·105	2·853	0·203	8·54	3·892	3·005	2·656	0·269	0·213	3·621
	5·0	16·60	0·065	4·188	1·515	0·287	7·28	4·099	1·428	3·557	0·437	0·160	3·805

[a] μ, exponential growth rate (day^{-1}); LPD, lag phase duration (days) calculated from the Gompertz model.

were smaller than the *F* table values (95% confidence). Derived parameters, μ (exponential microbial growth rate) and LPD (lag phase duration), from the two models did not show significant differences.

Table 1 shows the parameters obtained from each model and also the derived parameters μ and LPD. Propagation of errors (Himmelblau 1970) was used to calculate the corresponding standard errors. Only derived parameters from the Gompertz equation are shown in Table 1 because average standard errors were low.

LPD values of the various microorganisms (Table 1) ranged from 3·8 to 6·2 days for control samples. *Lactobacillus* sp and Enterobacteriaceae were the microorganisms most affected by the addition of 0·023 and 0·065 mM UAC citric acid with LPD values of 10·2–12·9 days. The LPD values of the psychrotrophic microorganisms did not differ from that of the control in this range of citric acid concentrations. The addition of 0·065 UAC citric acid increased the LPD of *Pseudomonas* sp from 5·1 to 8·9 days. Ascorbic acid (0·302 mM UAC) extended the LPD of Enterobacteriaceae from 4·9 to 13·6 days. The effect was lower for *Pseudomonas* sp and *Lactobacillus* sp and non-significant for psychrotrophic microorganisms.

Values of μ obtained as derived parameters of the Gompertz equation are reported in Table 1, and values of μ and IR obtained from the slopes of the linear regressions, are shown in Table 2. Negative IR values were observed as the concentration of the acids increased showing a bacteriostatic and slightly bactericidal effect on the microorganisms studied. For the control system (Table 1), *Pseudomonas* sp and psychrotrophic microorganisms grew at the highest rates with μ values of 0·869 and 0·700 day^{-1} respectively, followed by *Lactobacillus* sp ($\mu = 0{\cdot}393$ day^{-1}) and Enterobacteriaceae ($\mu = 0{\cdot}234$ day^{-1}).

The addition of 0·065 mM UAC citric acid (pH = 5·0) had a significant inhibitory effect on the μ values of *Pseudomonas* sp and psychrotrophic microorganisms

TABLE 2
Slopes of the linear regression (day^{-1}) of microbial counts vs time for various preservative concentrations

Microorganism	*Acid*	*pH*	*Total concentration* (*mM*)	*UAC* (*mM*)	*Slope of linear regression* (day^{-1})
Lactobacillus	Ascorbic	4·5	52.08	14·80	−0·014
		4·4	78·12	25·50	−0·011
	Citric	4·5	24·03	0·63	0·015
		4·3	48·07	2·34	0·014
		3·7	72·11	14·80	0·007
Enterobacteriaceae	Ascorbic	5·0	26·04	2·91	0·013
		4·5	52·08	14·80	−0·036
		4·4	78·12	25·50	−0·060
	Citric	4·5	24·03	0·63	−0·008
		4·3	48·07	2·34	−0·015
		3·7	72·11	14·80	−0·038
Pseudomonas sp	Ascorbic	4·5	52·08	14·80	−0·043
		4·4	78·12	25·50	−0·063
	Citric	4·5	24·03	0·63	0·038
		4·3	48·07	2·34	0·003
		3·7	72·11	14·80	−0·032
Psychrotrophic microorganisms	Ascorbic	4·5	52·08	14·80	0·068
		4·4	78·12	25·50	0·051
	Citric	4·5	24·03	0·63	0·055
		4·3	48·07	2·34	−0·004
		3·7	72·11	14·80	−0·017

decreasing 4·3–4·4 times with respect to control values; μ values of Enterobacteriaceae diminished only 2 times.

At the same pH value (pH = 5) the addition of ascorbic acid produced higher inhibition on Enterobacteriaceae than on the other microorganisms studied. In this case a bacteriostatic effect was observed ($\mu = 0{\cdot}013$ day^{-1}, Table 2); the μ value of Enterobacteriaceae decreased 20 times with respect to the control system. Thus, pH alone, without the specification of the acid, does not explain the inhibitory effect.

Figures 2(a)–(d) show the effects of undissociated citric and ascorbic concentration on the μ and IR values of Enterobacteriaceae, *Lactobacillus* sp, psychrotrophic microorganisms and *Pseudomonas* sp, in the concentration range studied (logarithmic scale). Bars indicate the corresponding standard errors of the means that were evaluated by error propagation (Himmelblau 1970).

In all the cases studied, an increase of undissociated acid concentration produced a decrease in μ values (Fig 2(a)–(d)). A critical UAC concentration was defined as that producing $\mu = \text{IR} = 0$. In the case of ascorbic acid, IR = 0 was reached at 2·9 mM for Enterobacteriaceae, 5 mM for *Pseudomonas* sp, 10 mM for *Lactobacillus* sp and a higher concentration than 25·5 mM UAC for psychrotrophic microorganisms. In the case of citric acid, the critical UAC was approximately 0·25 mM for Enterobacteriaceae, 0·40 mM for *Lactobacillus* sp and 2·30 mM for the psychrotrophic microorganisms and *Pseudomonas* sp. This shows that the critical concentration values of both acids were lowest for Enterobacteriaceae.

For UAC concentrations lower than 3 mM, Fig 2 shows, for all the microorganisms tested, higher μ values with ascorbic acid than with citric acid. Thus, citric acid produced a stronger inhibitory action on μ values at low concentrations; however at higher concentrations (>10 mM) both acids had similar effects on μ values.

Inhibitory effect of the acids

In order to compare the effects of the acids with respect to the control sample, an inhibitory effect on μ, $(\text{IE})_\mu$ was calculated as follows:

$$(\text{IE})_\mu = 1 - (\mu_{\text{treated}}/\mu_{\text{control}}) \quad \textbf{(6)}$$

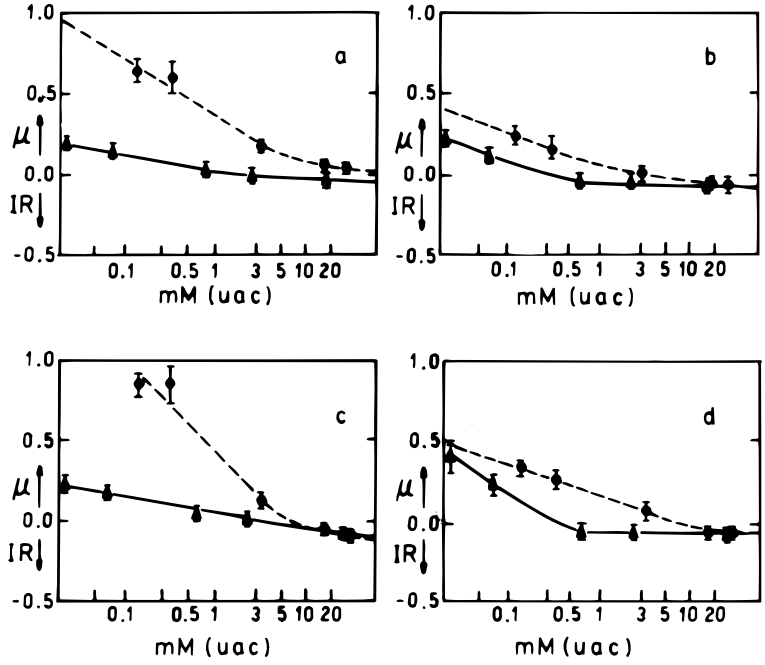

Fig 2. Values of exponential growth rate (μ) and inactivation rate (IR) for the various microorganisms as a function of undissociated acid concentration: (a) psychrotrophic microorganisms; (b) Enterobacteriaceae; (c) *Pseudomonas* sp; (d) *Lactobacillus* sp; ———, citric acid; – – – –, ascorbic acid.

where $\mu_{treated}$ and $\mu_{control}$ are specific exponential growth rate constants (days^{-1}), for treated and control samples, respectively. When a lethal effect was observed, μ was replaced by the inactivation rate (IR) which has negative values. $(IE)_\mu$ is 1 when microorganisms are in lag phase ($\mu = 0$), reaches zero when the treated and control samples show the same growth rate and is greater than 1 when lethal action on the bacteria is observed ($IR < 0$).

$(IE)_\mu$ shows the effect of preservatives on the growth rate and inactivation rate, but it does reflect the effect on lag phase duration (LPD). Thus, in a similar way, an inhibitory effect for the duration of the lag phase was defined:

$$(IE)_{LPD} = 1 - (LPD_{control}/LPD_{treated}) \qquad (7)$$

where $LPD_{control}$ and $LPD_{treated}$ are lag phase durations (days), for treated and control samples, respectively.

$(IE)_{LPD}$ is zero when an antimicrobial effect is not observed and the microorganisms in treated samples have the same LPD as in the control; $(IE)_{LPD}$ equals one when the microorganisms remain in the lag phase, that is, $LPD_{treated}$ is much higher than $LPD_{control}$.

Table 3 shows that the effects of undissociated citric and ascorbic acid concentrations on both inhibition indices, $(IE)_\mu$ and $(IE)_{LPD}$), for the microorganisms

TABLE 3
Inhibitory effect of undissociated citric and ascorbic acids $(IE)_\mu$ and lag phase duration $(IE)_{LPD}$ of the microorganisms

Preservative	*UAC* (*mM*)	*Enterobacteriaceae*		*Pseudomonas sp*		*Lactobacillus*		*Psychrotrophic micr*	
		$(IE)_\mu$	$(IE)_{LPD}$	$(IE)_\mu$	$(IE)_{LPD}$	$(IE)_\mu$	$(IE)_{LPD}$	$(IE)_\mu$	$(IE)_{LPD}$
Citric acid	0·023	0·06	0·51	0·73	0·26	nd	0·45	0·69	nd
	0·065	0·44	0·59	0·76	0·45	0·27	0·52	0·76	nd
	0·634	1·03	1	0·96	1	0·96	1	0·92	1
	2·340	1·06	1	0·99	1	0·96	1	1·01	1
	14·80	1·16	1	1·04	1	0·98	1	1·03	1
Ascorbic acid	0·125	nd	0·44	nd	nd	0·07	0·09	0·05	0·05
	0·302	0·32	0·64	nd	nd	0·25	0·04	0·11	0·02
	2·910	0·94	1	0·85	0·35	0·72	0·25	0·74	0·07
	14·80	1·15	1	1·05	1	1·04	1	0·89	1
	25·50	1·25	1	1·07	1	1·03	1	0·93	1

nd: effect not detected.

tested. Citric acid was the strong inhibitor with high values of $(IE)_{\mu}$ and $(IE)_{LPD}$ at low undissociated acid concentrations (<0·065 mM). In the range of 0·023 to 0·065 mM UAC citric acid was the most effective in reducing μ against *Pseudomonas* sp and psychrotrophic microorganisms, with the highest values of $(IE)_{\mu}$. However, $(IE)_{LPD}$ values were practically zero for psychrotrophic microorganisms at low concentrations of citric acid (<0·065 mM UAC). The highest $(IE)_{LPD}$ value was observed for Enterobacteriaceae in the concentration range studied.

The addition of ascorbic acid in concentrations higher than 0·302 mM UAC produced larger $(IE)_{\mu}$ and $(IE)_{LPD}$ values for Enterobacteriaceae than for the other microorganisms.

At UAC values higher than 0·634 mM citric acid and 14·80 mM ascorbic acid, both indices $(IE)_{\mu}$ and $(IE)_{LPD}$ were approximately 1 for all the microorganisms.

CONCLUSIONS

The effects of undissociated ascorbic and citric acid concentration on microbial growth parameters were analysed using linear and non-linear models (Gompertz and logistic) to fit microbial counts in a model system of potato homogenate.

For UAC concentrations lower than 3 mM, μ values of all the microorganisms tested were higher with ascorbic acid than with citric acid; however, at higher concentrations (>10 mM) both acids had similar effects on μ values.

The addition of 0·065 mM UAC citric acid (pH = 5·0) had a significant inhibitory effect on μ values of *Pseudomonas* sp and psychrotrophic microorganisms. At the same pH value (ie pH = 5) the addition of ascorbic acid produced higher inhibition of Enterobacteriaceae than of the other microorganisms studied. Thus, pH alone, without the specification of the acid, does not explain the inhibitory effect.

Pseudomonas sp were the microorganisms most inhibited by citric acid. Ascorbic acid, at low UAC concentrations (0·302 mM UAC) produced higher inhibition of Enterobacteriaceae than of the other microorganisms. For UAC concentrations lower than 3 mM, μ values of all the microorganisms tested were higher with ascorbic acid than with citric acid; however at higher concentrations (>10 mM) both acids had similar effects on μ values.

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