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Chemical Preservatives Action on Microbial Growth in a Model System of Refrigerated Prepeeled Potatoes

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ABSTRACT

The effect of different concentrations of citric acid and ascorbic acid (applied individually or in mixture's) on microbial growth in potato homogenate was analyzed and compared to the sodium bisulfite action during storage at 4°C in low gaseous permeability films. These experiments allowed one to simulate the behavior of prepeeled potatoes but with a known amount of added preservative to evaluate additive or synergic effects. Total viable microorganisms, *Enterobacteriaceae*, *Pseudomonas* sp., *Lactobacillus* sp., molds, yeasts, *Clostridium* sulfite reducers, psychrotropic microorganisms, and aerobic and anaerobic viable spores were analyzed during storage time. Inhibition indexes produced by the tested preservatives were calculated for the different microorganisms. Sodium bisulfite solutions (100 ppm) had no inhibitory effect. Concentrations of 3,500 ppm citric acid and 10,000 ppm ascorbic acid showed antimicrobial action as well as mixtures of citric acid/ascorbic acid of the following compositions (in terms of total acids concentration): 2,700/2,000, 3,500/2,000, and 2,700/3,000 ppm. A higher effect on *Enterobacteriaceae* was observed in comparison with other microorganisms. The apparent synergic effect of these acids when they were applied together was demonstrated to be actually an additive effect when concentrations of undissociated acid in the mixtures were considered instead of total concentration.

The risks of bacterial contamination and material spoilage are increased when vegetables are prepared for "ready to use". Bacteria are generally unable to penetrate the intact surface of the plant tissue, but the situation changes when the product is peeled. Consequently, addition of preservatives, packaging, and refrigerated storage conditions that would inhibit bacterial spoilage of prepared vegetables will increase the shelf life of the product.

Sodium and potassium sulfite, bisulfite and metabisulfite, which are very effective to prevent food decoloration due to enzymatic browning, have also a very useful antimicrobial activity. Packaging in films of low gaseous permeability have been used to reduce the required bisulfite levels (*1,6*). Previous work (*7*) has shown that the simultaneous effect of sodium bisulfite concentrations and vacuum packaging has a positive interaction on microbial growth. Thus, a diminution of the growth rate and an increase of the lag-phase duration can be clearly observed. It has been reported recently that sulfite may have adverse effects on sensitive persons; citric and ascorbic acids could potentially replace sulfiting agents (*2,5,10,11,14*).

The antimicrobial effect of ascorbic and citric acids constitutes an attractive alternative because these acids appear naturally in many foods; they are an essential nutrient and have been generally recognized as safe.

The commercial product (prepeeled potato) is not a good system to study the effect of preservative concentration on microbial growth. Such concentration is modified on the surface by diffusion into the tissue. Thus, it would be necessary to use a model system, as close as possible to the commercial product, with a well-known amount of preservative.

The objectives of the present work were: i) to analyze the effect of citric and ascorbic acids, individually applied or in combination, on microbial growth in a model system constituted by a potato homogenate and ii) to compare the effectivity of these acids to sodium bisulfite in the selected model system during refrigerated storage (4°C) in a plastic film of low gaseous permeability.

MATERIALS AND METHODS

Potato samples (Solanum tuberosum, Kennebec variety from Balcarce, Argentina), stored for 2 month 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. They were placed in test tubes, and chemical preservatives in known concentrations were added; this allowed one to analyze the inhibitory effect of each of the preservatives. The potato water content was taken into account to calculate the concentration of added preservative. The control samples consisted of the same homogenate without preservatives addition. Table 1 shows the tested chemical preservatives and the corresponding concentrations ranges. After addition of the preservatives, a Vortex Mixer (50% sample volume, 50% head volume) was used to obtain uniform concentration. Test tubes were packed in EVA/SARAN/EVA film (oxygen permeability = 37 cm^3m^{-2} atm^{-1} day^{-1}) with partial gaseous evacuation in a Minidual equipment Model MW 4980 (Schocolnik SAIC, Buenos Aires). Manometric pressure in the vacuum chamber was 4.5 mm Hg; storage was carried out at 4°C.

Atmosphere analysis

To determine the gaseous microatmosphere composition inside the test tubes, gas samples (25 µl) were periodically removed from duplicate tubes of potato homogenate and analyzed in a gas

TABLE 1. *Concentration of undissociated citric, ascorbic acids, and SO_2 in the potato homogenate.*

Preservatives	Total concentration of added preservatives (ppm)	pH	Concentration of the undissociated preservatives (ppm)		
			SO_2	Citric acid	Ascorbic acid
Sodium bisulfite pK1 = 1.75 pK2 = 7.21	66	6.1	0.0027		
	100	6.0	0.0052		
	219	5.9	0.0147		
Citric acid pK1 = 3.14 pK2 = 4.77 pK3 = 6.69	2,700	5.1		4.7	
	3,500	5.0		13	
	5,000	4.5		132	
	10,000	4.3		487	
	15,000	3.7		3,082	
Ascorbic acid pK1 = 4.00 pK2 = 11.79	2,000	6.0			24
	3,000	5.8			58
	5,000	5.0			559
	10,000	4.5			2,846
	15,000	4.4			4,905
Citric/ ascorbic mixtures	2,700/2,000	4.6		51	480
	2,700/3,000	4.4		97	981
	3,500/2,000	4.2		224	884
Sodium bisulfite/ citric acid mixture	66/2,500	5.2	0.023	5.7	

chromatograph GC-6A (Shimadzu, Japan) with thermal conductivity detector and a Data Processor Chromatopac C-R 1A (Shimadzu, Japan).

The technique (*9*) was modified by connecting a silica gel column (30-60) of 1-m length which operated at 117°C within the oven of the gas chromatograph and a molecular sieve column 5A which operated at 0°C. Under the described experimental conditions, the average retention times for the different tested gases were CO_2:0.82 min, O_2:1.32 min, N_2:3.48 min. All the gas concentrations were expressed as percent (vol/vol).

Determination of sodium bisulfite and citric and ascorbic acids concentrations in potato homogenate

Considering that concentration of added preservatives may change during time, owing to enzymatic reaction, evaluation of these concentrations during storage period was performed.

Sodium bisulfite concentration was determined by Hart and Fisher (*8*) and Ross and Treadway (*16*) methods.

Citric acid was determined by two methods: i) Titratable acidity according to method 22058 of the Association of Official Analytical Chemists (AOAC) (*3*), consisting of titration with 0.1 N NaOH using phenolphtalein as the indicator. The extraction of citric acid was performed according to AOAC method 22-008 (*3*). ii) Using the Enzymatic Boehringer Kit for Food Analysis (Cat. No. 139076 Boehringer Mannheim GmbH Biochemica).

L-ascorbic acid was determined by two methods: i) Reduction of 2-6 diclorophenolindophenol dye by ascorbic acid in acid solution. The sample was blended with 0.5 % oxalic acid (*13*). ii) Using the Enzymatic Boehringer Kit for Food Analysis (Colorimetric Method, Cat. No. 409677).

Microbiological analysis

The composition of the microflora in untreated potatoes at the beginning and end of the storage period was determined analyzing the colonies that grew in selective media as was reported in a previous study (*7*).

During storage the following analyses were performed: i) Enumeration of total mesophilic aerobic microorganisms: On 1 g of homogenate, 1 ml of the necessary dilution in 0.1% sterile peptone water was inoculated in plate count agar (Merck) (pour plate procedure) with incubation at 30°C for 2 d. Results were expressed as log CFU/g. ii) Psychrotropic microorganism: 1 m of the necessary dilutions was inoculated in plate count agar and incubated at 4°C for 7 d. iii) *Enterobacteriaceae* counts: 0.1 ml of the necessary dilutions were inoculated in Caso agar (casein-peptone soymeal-peptone broth) (Merck) overlayed with red bile violet dextrose agar (Merck), melted and cooled at 45°C. It was incubated at 37°C for 18-24 h. iv) *Pseudomonas* sp.: It was carried out by inoculating 0.1 ml of the necessary dilutions in Masurovsky agar (*12*) with incubation at 30°C for 2 d. v) *Lactobacillus* sp.: 0.1 ml of the necessary dilution was inoculated in Caso agar (Merck) overlayed with Rogosa agar (*Lactobacillus* selective agar) (*15*) (Merck), melted and cooled at 45°C with incubation at 30°C for 3 d in anaerobic conditions. vi) Enumeration of aerobic and anaerobic viable spores: The first homogenate dilution (10^{-1}) was heated at 80°C for 10 min, then 1 ml was inoculated into plate count agar (pour plate procedure). It was incubated at 30°C in aerobic and anaerobic conditions. vii) Enumeration of *Clostridium* sulfite reducers: It was carried out according to the most probable number technique in a series of five tubes with 5 g of homogenate heated at 80°C for 10 min. SPS agar (Merck) was used, melted and cooled. The anaerobic incubation was performed at 30°C for 5 d. viii) Molds and yeasts: 0.1 ml of the dilutions was inoculated in OGC agar (Merck) (Oxytetracycline-glucose-cloranphenicol) with incubation at 25°C for 7 d.

The inhibition index of each microorganism for the different preservative concentrations was defined as follows:

$$\text{Inhibition index (II)} = 1 - \frac{\log(N/No)\ \text{treated}}{\log(N/No)\ \text{untreated}} \quad (1)$$

where log(N/No) of the treated and untreated samples was evaluated at the end of the storage time (20 d).

Additive effect is reported when the combined effect is equal to the sum of the effects observed with the two agents tested separately or equal to that of the most active agents in combination (*4*).

Synergistic effect is observed when the effect obtained with the combination of preservatives is significantly greater ($p < 0.05$) than the sum of the effects produced by the tested agents separately.

RESULTS AND DISCUSSION

Composition of gaseous atmosphere

Chromatograms showed a rapid evolution of CO_2, from an initial concentration <2% to a final concentration of 20-30%, a result of vegetable respiration and microbial growth.

Final oxygen concentration (15-20%) permitted the growth of aerobic and microaerobic bacteria.

Effect of preservatives addition on the growth of microorganisms

Figure 1a shows the effect of sodium bisulfite at different concentrations on the growth of total viable microorganisms. It can be observed that 66 and 100 ppm SO_2 in potato homogenate do not inhibit microbial growth reaching 10^6 CFU/g after 6 and 7 d of storage (end of shelf life). Nevertheless, 219 ppm SO_2 allowed microorganisms to be kept in lag phase during a storage time of 20 d. The pH of the homogenate was similar to the control (5.9-6.1). Microbial inhibition was also observed when a mixture of preservatives with lower sodium bisulfite concentration was used (2,500 ppm citric acid/66 ppm SO_2); in this case microbial counts and shelf life were comparable to those found with 219 ppm SO_2. This result indicated that adding acid, SO_2 concentration in potatoes, can be reduced to levels (*16*) which are close to those accepted as maximum values (50 ppm, United Kingdom) as was reported previously for the commercial product (*6,7*).

The addition of citric acid modified the pH of the homogenate, producing a displacement of the SO_2 equilibrium reactions towards the nondissociated species that shows antimicrobial effect.

Weak acids

Citric acid. The inhibitory effect on total microorganisms, which is produced by an increase of citric acid concentration, is evidenced by a decrease of the microbial growth rate and by an increase of the lag phase (Fig. 1b). Concentrations of 3,500 ppm citric acid (pH of the homogenate = 5.0) maintained the counts at levels lower than 10^6 CFU/g during a storage of 20 d. Higher concentrations (5,000, 10,000, and 15,000 ppm) kept microorganisms in lag phase during a storage time of 20 d; however, in the upper zone, the homogenate growth of molds and yeasts was detected by visual observation.

Ascorbic acid. Ascorbic acid addition in concentrations varying from 930 to 5,000 ppm did not show inhibitory effect on the growth of total microorganisms, while the use of 10,000 ppm (pH = 4.5) produced an effective inhibition (Fig. 1c). In this case, the shelf life was 20 d with counts of 3×10^5 CFU. At higher concentrations (15,000 ppm, pH = 4.4),

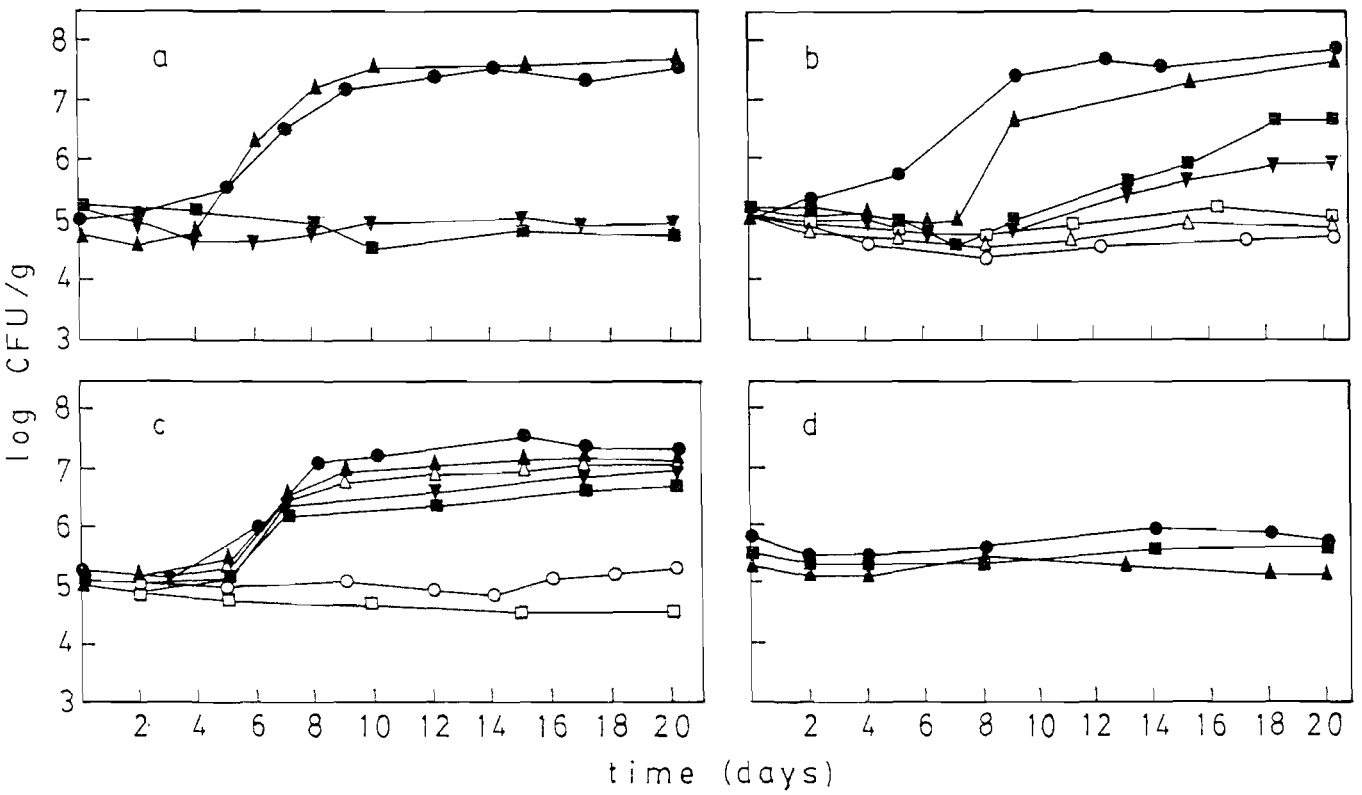

Figure 1. *Effect of different preservatives on total aerobic viable microorganisms in potato homogenate. a)SO_2 concentration: ■ 219 ppm, ● 100 ppm, ▲ 66 ppm. SO_2/citric acid concentration: ▼ 2,500/66 ppm. b) Citric acid: ● control; ▲ 2,500 ppm; ■ 2,700 ppm; ▼ 3,000 ppm; ❑ 5,000 ppm; Δ 10,000 ppm; ○ 15,000 ppm. c) Ascorbic acid: ● control; ▲ 930 ppm; Δ 2,000 ppm; ▼ 3,000 ppm; ■ 5,000 ppm; ○ 10,000 ppm; ❑ 15,000 ppm. d) Citric acid/ascorbic acid mixtures: ● 2,700/2,000 ppm; ■ 3,500/2,000 ppm; ▲ 2,700/3,000 ppm. Each value represents an average of duplicate determinations.*

growth of molds was observed in the system.

Citric/ascorbic mixtures. The mixtures of citric/ascorbic acids (2,700/2,000, 2,700/3,000, and 3,500/2,000 ppm) kept total microbial counts in lag phase (Fig. 1d).

Undissociated fraction of the weak acids

The undissociated concentration of the weak acids can be calculated by the following equations:
For a diprotic acid (ascorbic acid)

$$[AH_2] = \frac{Ca[H^+]^2}{[H^+]^2 + [H^+]\,K_1 + K_1K_2} \qquad (2)$$

For a triprotic acid (citric acid)

$$[AH_3] = \frac{Ca[H^+]^3}{[H^+]^3 + [H^+]^2K_1 + [H^+]K_1K_2 + K_1K_2K_3} \qquad (3)$$

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

Equations 2 and 3 were applied to calculate the undissociated concentrations of ascorbic and citric acid at different pH (Table 1) fitting the values of K_1, K_2, and K_3 obtained from pK values showed in the same table. As can be observed for the same concentration of total acid (5,000 ppm of citric or ascorbic acid applied individually) the corresponding concentration of undissociated citric acid is 132 ppm compared with 559 ppm of ascorbic acid. Nevertheless, the microbial inhibition produced by the citric acid is higher than by the ascorbic acid (Fig. 1b and c). For the same pH values of the homogenate (pH = 5.0), the corresponding concentration of undissociated citric acid was 13 ppm, compared with 559 ppm of ascorbic acid. However, this concentration of citric acid was quite effective to avoid exceeding 10^6 CFU/g total microbial counts during storage. A greater microbial growth was clearly observed with 559 ppm of undissociated ascorbic acid since counts were near 10^7 CFU/g at the end of the storage. This indicates that pH alone does not explain the inhibitory effects observed for both acids.

When both acids were applied together, the pHs of the three systems decrease to 4.6, 4.4, and 4.2, respectively, producing a displacement of the species towards the undissociated form.

The undissociated fraction of each acid increased 20 to 30 times in relation to those present in the system which was treated with the preservatives separately (Table 1).

Effect of preservatives on the different components of the microbial flora

The microbial flora is mostly formed by *Enterobacteriaceae, Pseudomonas* sp., *Lactobacillus* sp., psychrotropic microorganisms, molds, and yeasts. The effect of the analyzed preservatives on these microorganisms was studied in order to observe their sensitivity to the tested acids and the antimicrobial effects when they were applied together. Results were compared with a treatment of 100 ppm SO_2, residue which is higher than the maximum of 50 ppm allowed internationally for raw peeled potatoes (*17*).

Molds and yeasts

Mean counts of molds and yeasts in the control sample homogenate were 10^4 and 5 x 10^5 CFU/g at the beginning and end of the storage, respectively. The microscopic observation allowed the identification of the genera *Rodotorula* as predominant yeasts and of *Alternaria, Penicillium, Aspergillus,* and *Cladosporium* as typical molds.

The addition of citric acid (2,700-3,500 ppm) and ascorbic acid (2,000-3,000 ppm) individually had inhibitory effect; microbial counts did not exceed 10^5 CFU/g during storage.

The use of preservative mixtures (citric/ascorbic) did not produce important changes during storage because the counts started with 2 x 10^4 CFU/g and finished with 5 x 10^4 CFU/g. These results indicated that the mixtures of preservatives did not produce on molds and yeasts a greater inhibition than that caused by the acid when used separately.

The addition of 100 ppm SO_2 did not show inhibitory effect on the growth of molds and yeasts (8 x 10^4 CFU/g).

Aerobic and anaerobic viable spores

Aerobic and anaerobic viable spore counts did not change appreciably during the storage of the homogenate treated with citric acid (2,700-3,500 ppm), ascorbic acid (2,000-3,000 ppm), and with SO_2 (100 ppm); counts ranged between 10 to 10^2 CFU/g. Citric/ascorbic mixtures did not show significant differences in relation to the samples treated with the individual preservatives.

Clostridium *sulfite reducers microorganisms*

Counts of sulfite reducers microorganisms according to the most probable number technique were 0.34-0.37 g^{-1}; this would indicate that it is a product of low risk. No significant differences were observed between the homogenates treated with citric acid (2,700-3,500 ppm), ascorbic acid (2,000-3,000 ppm), SO_2 (100 ppm), and in the mixtures of citric/ascorbic.

Pseudomonas *sp.*

The addition of 2,700 and 3,500 ppm of citric acid permitted the growth of *Pseudomonas* sp. which reach 3 x 10^6 and 8 x 10^5 CFU/g, respectively, at the end of storage. Concentrations of 5,000 and 10,000 ppm maintained microorganisms in lag phase; 15,000 ppm has bactericidal effect (Fig. 2a).

With 2,000 and 3,000 ppm of ascorbic acid, counts reached 10^7 and 10^6 CFU/g, respectively, at the end of the storage; 5,000 ppm ascorbic acid produces inhibition on those microorganisms which reach 3 x 10^5 CFU/g. Higher concentrations kept counts in lag phase during storage (Fig. 2b).

Sulfur dioxide (100 ppm) did not have inhibitory effect on *Pseudomonas* sp. because the growth rate was similar to that of the control, reaching 10^7 CFU/g at the end of the storage. Effect of mixtures of citric/ascorbic did not differ among each other, and the counts remained in lag phase; they were 3- and 4-log cycles lower than the control at the end of the storage period (Fig. 2c).

Enterobacteriaceae

The use of 2,700 and 3,500 ppm of citric acid did not

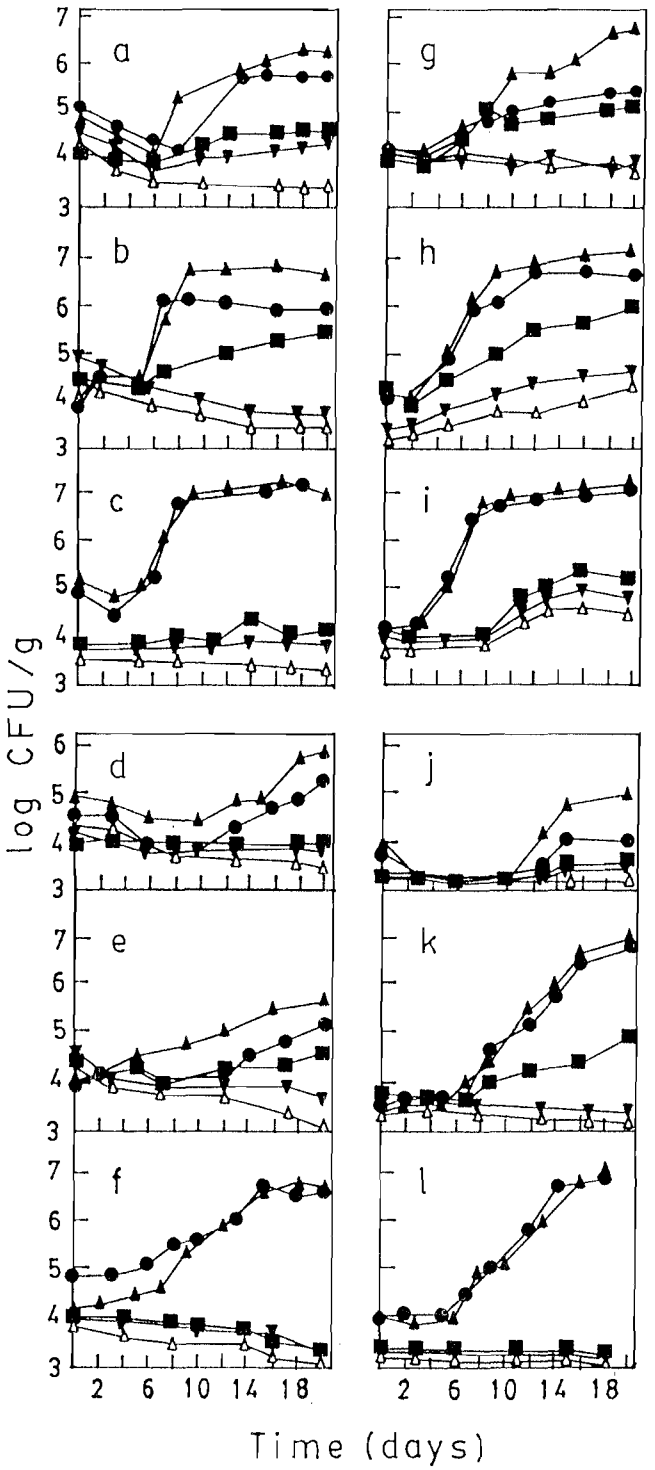

Figure 2. *Influence of chemical preservatives addition on microbial flora. (a, b, c)* Enterobacteriaceae: *(a) citric acid concentration: ▲ 2,700 ppm; ● 3,500 ppm; ■ 5,000 ppm; ▼ 10,000 ppm; Δ 15,000 ppm. (b) ascorbic acid concentration: ▲ 2,000 ppm; ● 3,000 ppm; ■ 5,000 ppm; ▼ 10,000 ppm; Δ 15,000 ppm. (c) ● control; ▲ 100 ppm SO_2; citric acid/ascorbic acid mixtures: ■ 2,700/2,000 ppm; ▼ 3,500/2,000 ppm; Δ 2,700/3,000 ppm. (d, e, f)* Pseudomonas *sp.: (d) citric acid concentration: ▲ 2,700 ppm; ● 3,500 ppm; ■ 5,000 ppm; ▼ 10,000 ppm; Δ 15,000 ppm. (e) ascorbic acid concentration: ▲ 2,000 ppm; ● 3,000 ppm; ■ 5,000 ppm; ▼ 10,000 ppm; Δ 15,000 ppm. (f) ● control; ▲ 100 ppm SO_2; citric acid/ascorbic acid mixtures: ■ 2,700/2,000 ppm; ▼ 3,500/2,000 ppm; Δ 2,700/3,000 ppm. (g, h, i) Psychrotropic microorganisms: (g) citric acid concentration: ▲ 2,700 ppm; ● 3,500 ppm; ■ 5,000 ppm; ▼ 10,000 ppm; Δ 15,000 ppm. (h) ascorbic acid concentration: ▲ 2,000 ppm; ● 3,000 ppm; ■ 5,000 ppm; ▼ 10,000 ppm; Δ 15,000 ppm. (i) ● control; ▲ 100 ppm SO_2; citric acid/ascorbic acid mixtures: ■ 2,700/2,000 ppm; ▼ 3,500/2,000 ppm; Δ 2,700/3,000 ppm. (j, k, l)* Lactobacillus *sp.: (j) citric acid concentration: ▲ 2,700 ppm; ● 3,500 ppm; ■ 5,000 ppm; ▼ 10,000 ppm; Δ 15,000 ppm. (k) ascorbic acid concentration: ▲ 2,000 ppm; ● 3,000 ppm; ■ 5,000 ppm; ▼ 10,000 ppm; Δ 15,000 ppm. (l) ● control; ▲ 100 ppm SO_2; citric acid/ascorbic acid mixtures: ■ 2,700/2,000 ppm; ▼ 3,500/2,000 ppm; Δ 2,700/3,000 ppm. Each value represents an average of duplicate determinations.*

show significant differences in the growth rate. Counts reached 10^6 and 2.5 x 10^5 CFU/g, respectively, at the end of the storage. Higher concentrations such as 5,000, 10,000, and 15,000 ppm kept the counts in lag phase, without exceeding 10^4 CFU/g at the end of the storage (Fig. 2d).

Concentrations of 2,000 and 3,000 ppm ascorbic acid led to 5.6 x 10^5 and 1.5 x 10^5 CFU/g, respectively, after a storage of 20 d. Higher concentrations had inhibitory effect on these microorganisms (Fig. 2e); 100 ppm SO_2 did not produce inhibition (the behavior was similar to the control), leading to 10^7 CFU/g after a storage time of 20 d. The three assayed mixtures of both preservatives had inhibitory effect on microbial growth. The counts were 3- to 3.5-log cycles lower than the control; at the end of the storage, microbial counts varied from 1 x 10^3 to 5 x 10^3 CFU/g (Fig. 2f).

Psychrotropic microorganisms

Psychrotropic microorganisms behave similarly to *Pseudomonas* sp., because with 2,700 ppm citric acid they reached counts very close to 8 x 10^6 CFU/g. A lower growth rate was observed with 3,500 and 5,000 ppm; the counts vary from 6 x 10^5 to 2 x 10^5 CFU/g, respectively. Concentrations of 10,000 and 15,000 ppm kept microorganisms in lag phase during storage (Fig. 2g); 2,000 and 3,000 ppm ascorbic acid did not have an inhibitory effect on psychrotropic microorganisms. With 5,000 ppm, microorganisms grew more slowly reaching 10^6 CFU/g after 20 d. Higher concentrations (10,000 and 15,000 ppm ascorbic) produced inhibition; counts of 5 x 10^4 CFU/g (Fig. 2h) were observed at the end of the storage.

Sulfur dioxide (100 ppm) did not have an inhibitory effect on these microorganisms, in that they reached 10^7 CFU/g after 20 d of storage. A very slight microbial growth was observed in the tested mixtures of citric/ascorbic preservatives; final counts varied from 5 x 10^5 to 10^4 CFU/g and were 2- or 3-log cycles lower than those of the control (Fig. 2i).

Lactobacillus *sp.*

Final values of 10^5 and 10^6 CFU/g were found with 2,700 and 3,500 ppm citric acid, respectively, after 20 d of storage; microorganisms remained in lag phase with 5,000, 10,000, and 15,000 ppm, since these microorganisms were those most strongly inhibited by citric acid (Fig. 2j).

Ascorbic acid (2,000 and 3,000 ppm) did not show inhibitory effect on *Lactobacillus* sp. which reach counts close to 10^7 CFU/g at the end of the storage. Lower growth rate and final counts of 10^5 CFU/g were observed with 5,000 ppm ascorbic acid. With 10,000 and 15,000 ppm counts remained in lag phase during storage (Fig. 2k).

Sulfur dioxide (100 ppm) did not show inhibitory effect

on these microorganisms which grow similarly to those of the control with final count of 10^7 CFU/g after 20 d of storage.

The three studied citric/ascorbic mixtures showed that the *Lactobacillus* sp. were inhibited; counts of 3 x 10^2 and 10^3 CFU/g were obtained at the end of the storage, which were 3.5- to 4-log cycles lower than those of the control (Fig. 2*l*).

The described results made it possible to presume the existence of a synergistic effect of the preservatives acting together on microbial inhibition. Such effect was observed when preservatives acted together in relation to microbial counts obtained by the application of each preservative separately.

In order to evaluate if it is actually synergism, another parameter such as the inhibition index is very appropriate to study the behavior of the applied acids upon each of the studied microorganisms.

Inhibition index

Inhibition indexes (II) for the different microorganisms (*Pseudomonas* sp., *Enterobacteriaceae*, psychrotropic microorganisms and *Lactobacillus* sp.) (Table 2) were evaluated according to equation (1) at the end of storage, with experimental data obtained from Fig. 2 (a to *l*).

When the inhibition index was close to one, microorganisms were kept in lag phase; if it was higher than 1, bactericidal effect was produced, and if it was close to zero microbial growth was observed.

The II of the citric and ascorbic acid mixtures for the different analyzed microorganisms were significantly greater than the sum of those obtained by applying the preservatives separately. This would indicate synergism in the combined action of both preservatives on microbial inhibition. Nevertheless, this affirmation might not be correct since comparison of systems with the same total concentration of citric and ascorbic acids, but different undissociated fractions, was made (Table 1).

Considering that the antimicrobial action of weak acids is generally attributed to the undissociated fraction, the II for the different components of the microbial flora as a function of the undissociated concentrations of citric and ascorbic acids, respectively, were plotted in Fig. 3 (a to h) from experimental data of Fig. 2 (a to *l*). Linear correlations between both parameters are clearly observed. *Pseudomonas* sp. and *Enterobacteriaceae* were more strongly inhibited by citric acid than by ascorbic acid at low concentrations. At high concentrations ascorbic acid produced stronger inhibition on both microorganisms.

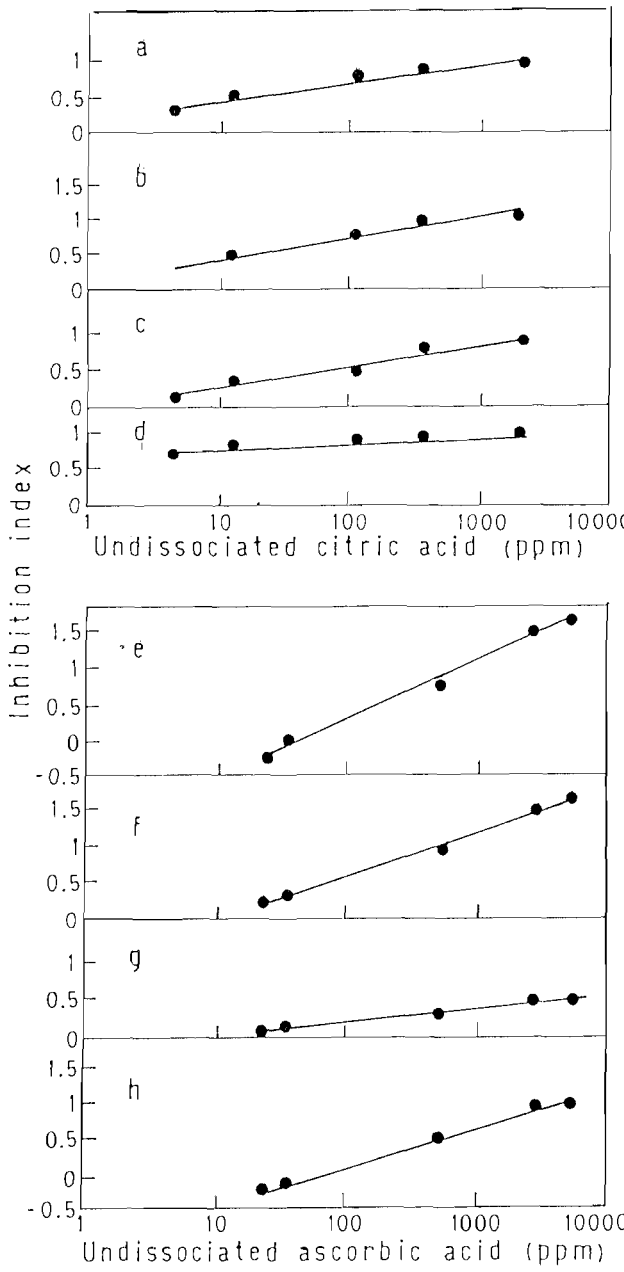

Figure 3. *Inhibition index as a function of undissociated acid concentration (ppm) (a, b, c, d) citric acid: (a)* Pseudomonas *sp.; (b)* Enterobacteriaceae; *(c) Psychrotropic microorganisms; (d)* Lactobacillus *sp.. (e, f, g, h) ascorbic acid; (e)* Pseudomonas *sp.; (f)* Enterobacteriaceae; *(g) Psychrotropic microorganisms; (h)* Lactobacillus *sp. Each value represents an average of duplicate determinations.*

TABLE 2. *Inhibition index of microbial growth for different total acid concentrations.*

Total acid concentration (ppm)	*Pseudomonas*	Psychrotropic	*Enterobacteriaceae*	*Lactobacillus*
Citric 2,700	0.36	0.07	0.50	0.50
Citric 3,500	0.37	0.50	0.66	0.66
Ascorbic 2,000	-0.16	0.06	0.15	-0.18
Ascorbic 3,000	0.03	0.15	0.30	-0.09
Citric/Ascorbic				
2,700/3,000	0.98	0.75	1.30	0.99
3,500/2,000	1.00	0.70	1.26	0.99
2,700/2,000	0.86	0.66	1.22	0.98

TABLE 3. *Inhibition index of microbial growth for different undissociated acid concentrations (obtained from Fig. 3 and 4).*

Microorganisms	Total concentration of mixtures (ppm)					
	2,700 citric	2,000 ascorbic	2,700 citric	3,000 ascorbic	3,500 citric	2,000 ascorbic
	Undissociated acid concentration (ppm)					
	51	480	97	980	224	884
Pseudomonas sp.	0.72	0.82	0.77	1.10	0.88	0.91
Psychrotropic	0.50	0.37	0.62	0.42	0.72	0.40
Enterobacteriaceae	0.73	1.05	0.76	1.25	0.90	1.22
Lactobacillus sp.	0.87	0.55	0.88	0.63	0.90	0.66

Psychrotropic microorganism were more strongly inhibited by the undissociated citric acid than by ascorbic acid. *Lactobacillus* sp. were more inhibited by citric acid at low concentrations, but at higher concentrations they were inhibited similarly by citric and ascorbic acids.

In order to evaluate synergic effect, comparisons of the II have to be made on the basis of the same undissociated acid concentrations and not on the basis of total concentrations. Table 3 shows inhibition indexes (II) corresponding to undissociated citric and ascorbic acid concentrations that were obtained from Fig. 3 (a to h) for conditions reported in Table 1; these are the actual conditions in the mixtures.

For example, in order to analyze the presence of synergism in the mixture (2,700 ppm total citric acid-2,000 ppm total ascorbic acid) inhibition indexes for the different microorganisms obtained from Table 2 were compared with those produced by 51 ppm of undissociated citric acid and 480 ppm of undissociated ascorbic acid (Table 3). In the case of *Pseudomonas* sp., II of the mixture citric/ascorbic (2,700/3,000 ppm) is 0.96 that is not greater than the sum of 0.77 + 1.10.

Thus, once the comparison was established on the basis of undissociated concentrations, it can be concluded that none of the three assayed mixtures produced synergistic effect on the microbial inhibition of the tested microorganisms. This is due to the fact that the II obtained when preservatives were applied in combination were not significantly higher than the sum of the II produced by the corresponding undissociated acids. In fact, the effect was additive, since the II obtained when preservatives are used together, were the same as the II produced by the acid which is more active in the combination. It can also be observed that a higher inhibitory effect appeared on *Enterobacteriaceae* which were the most sensitive microorganism to the three mixtures of the tested preservatives.

CONCLUSIONS

- Sodium bisulfite in 100 ppm was not inhibitory for any of the microorganisms growing in this system.
- Citric and ascorbic acids were good antimicrobial agents and also were the mixtures of both preservatives.
- The fraction of undissociated acid was the effective antimicrobial species, which was 20 to 30 times greater in the preservatives mixture than it was in treatments with the acids used separately.
- No synergic but additive effect was observed on the microbial inhibition as it was expressed according to the concentration of undissociated acid in the mixture.
- The three mixtures of the assayed preservatives showed higher inhibition on *Enterobacteriaceae* with respect to the other microorganisms.

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REFERENCES

1. Anderson, E. E., and C. Zapsalis. 1957. Technique ups quality, shelf life of pre-peeled potatoes. Food Eng. 29:114-116.
2. Andres, C. 1983. NRA asks members to discontinue sodium bisulfite use. Food Processing 44:52.
3. Association of Official Analytical Chemists. 1984. Official methods of analysis, 14th ed. 22-008, 22-058. Association of Official Analytical Chemists, Washington DC.
4. Davidson, P. M., and M. E. Parish. 1989. Methods for testing the efficacy of food antimicrobial. Food Technol. 43:148-155.
5. Dziezak, T. 1986. Preservative: Antioxidants. Food Technol. 40:94-102.
6. Giannuzzi, L., N. Rodriguez, and N. Zaritzky. 1988. Influence of packaging film permeability and residual sulphur dioxide on the quality of pre-peeled potatoes. Int. J. Food Sci. Technol. 23:147-152.
7. Giannuzzi, L. and N. Zaritzky. 1990. Effect of sulphur dioxide on microbial growth in refrigerated pre-peeled potatoes packaged in plastic film. J. Food Agric. 51:369-379.
8. Hart, F. L., and H. J Fisher. 1971. Modern food analysis methods 17-13. Springer Verlag, New York.
9. Karel, M., P. Issenberg, L. Ronsivalli, and V. Jusin. 1963. Application of gas chromatograph to the measurement of gas permeability of packaging materials. Food Technol. 17:91-94.
10. Label, F. 1983. Sulfite alternatives. Food Processing 11:54.
11. Langdon, T. 1987. Preventing of browning in fresh prepared potatoes without the use of sulfiting agent. Food Technol. 41:64-67.
12. Masurovsky, E. B., S. A. Goldblith, and J. Voss. 1963. Differential medium for selection and enumeration of members of the genus *Pseudomonas*. J. Bacteriol 85:722-723.
13. Pointing, J. D. 1943. Extraction of ascorbic acid from plant material. Ind. Eng. Chem. Anal. Ed. 15:389-391.
14. Rice, J. 1983. Ascorbic/citric acid an answer to the sulfite questions. Food Processing 11:74.
15. Rogosa, M., J. A. Mitchell, and R. F Wiseman. 1951. A selective medium for the isolation of oral and faecal lactobacilli. J. Bacteriol. 62:132-133.
16. Ross, L. R., and R. M. Treadway. 1961. Factors affecting the sulfur dioxide uptake in sulfite pre-peeled potatoes. Am. Potato J. 38:9-13.
17. Statutory Instruments. 1971. Food and drugs. Composition and labelling SI N° 882. The preservatives in food (Amendment) regulations. United Kingdom.