Mathematical Modeling of Microbial Growth in Packaged Refrigerated Orange Juice Treated with Chemical Preservatives

S.C. ANDRÉS, L. GIANNUZZI, AND N.E. ZARITZKY

ABSTRACT: Microbial flora of refrigerated orange juice was analyzed during storage at 10 °C and the effects of the following factors were discussed: 1) the previous washing process of the orange peel, 2) the different levels of the added preservatives (citric acid, ascorbic acid, potassium sorbate, sodium benzoate), 3) the gaseous permeabilities of the packaging film. Gompertz equation was applied to model molds and yeasts growth for the different treatments and packaging conditions. The washing procedure with sodium hypochlorite extend 2 - 3 d the storage life of the juice (time to reach microbial counts of 10^6 CFU/ml) in both packaging films. The use of organic acids and potassium sorbate or sodium benzoate (1.66 - 6.94 mM) led to storage life values > 11 d in polyethylene and > 20 d in the low gaseous permeability film, maintaining good sanitary conditions.

Key Words: microbial growth, orange juice, packaging, chemical preservatives, Gompertz equation

Introduction

UNPASTEURIZED JUICES ARE MUCH MORE SUSCEPTIBLE TO microbial deterioration than juices pasteurized under normal time/temperature regimens. Unpasteurized citrus juices rely only upon low pH, cold temperature, scrupulous sanitation and GMP's to inhibit microbial contamination and proliferation during handling, storage and distribution.

Juices as fruit products or as ingredients have to be pasteurized or must receive an equivalent process that insures the production of juice free from pathogenic microorganisms due to outbreaks of foodborne illness by microorganisms as *E. coli* O157:H7, *Salmonella* and *Criptosporidium* (Matthys 1999).

Washing the orange peel with chlorinated water is recommended to remove soil from fresh products. If washing is done properly, microbial populations can be lowered (Ayhan and others1998).

Yeasts predominate in spoilage of acid fruit products because of their high acid tolerance and the ability of many of them to grow anaerobically. Yeasts, molds, and lactic acid bacteria have been implicated in spoilage of fruit juices (Deak and Beuchat1993).

Technological modifications in citrus processing could influence the frequency of mold spoilage in juices. The use of oxygen barrier containers allowed to reach much longer retail shelf life of chilled juices; reduced oxygen tension negatively affects mold growth rates, however, mycelia can grow under low oxygen conditions in vitro (Parish 1991).

Soft drinks and fruit preserves sometimes require the addition of chemical preservatives to improve their storage stability. These additives should be used carefully and only when there is a clear need to increase shelf life, prevent spoilage, or minimize the food-poisoning risk. Application of citric acid to prolong the storage life of peeled oranges has been reported by Pao and Petracek (1997). Infusion of fruits with citric acid solution (0.1, 0.25, 0.5, and 1.0% w/v) during the peeling process reduced the surface pH of the peeled fruits from 6.0 to values lower than 4.6 and extended their life in comparison with fruits infused with water only.

Predictive microbiology is a useful tool to determine shelf life of food products. The concept is based on the development of mathematical models that describe the influence of predominant controlling factors (such as temperature, pH, water activity, gaseous atmosphere, and preservatives) on the lag phase and growth rate of pathogenic and spoilage organisms. These predictions, however, can only be made through interpolation within the range of factors examined (Ross and McMeekin 1991). Kopelman and Rauchwerger (1984) successfully used kinetics data (both microbial and taste) for the prediction of microbial growth and for taste deterioration under variable time-temperature conditions (that is, simulation of a cold chain path).

The objectives of the present work were to analyze and to model microbial flora in natural unpasteurized orange juice during refrigerated storage at 10 °C and to study the effect of the following factors on the shelf life of the product: (i) previous washing process of the fruit peel, (ii) addition of preservatives to the juice (citric acid, ascorbic acid, potassium sorbate, sodium benzoate), (iii) use of packaging film with different gaseous permeabilities.

Materials and Methods

FRESH VALENCIA ORANGES WERE PURCHASED IN LOCAL MARkets and stored at 10 °C. The fruits were washed, and brushed manually; two washing procedures of the orange peel were tested: (1) with potable water or (2) with sodium hypochlorite solution (0.25 g/L) and finally with potable water. Oranges were cut in halves and squeezed to obtain the juice; all instruments and equipment were sanitized with 0.25 g/L chlorinated water (Ayhan and others 1998). Juice samples obtained from fruits submitted to the different peel washing procedures and concentrations of the added preservatives were indicated in Table 1. Microbial flora was analyzed in natural unpasteurized orange juice (samples A and B) and in treated samples (C to I). Sample C corresponds to a juice that was obtained from oranges whose peel was washed with sodium hypochlorite solution (0.25 g/L) and contains natural additives: citric acid, ascorbic acid, and sucrose. Selected levels of natural preservatives in sample C were determined from previous works by sensory analysis (Andrés and others 1999).

Table 1—Composition of the orange juice tested samples

Orange juice Sample	Peel washing procedure	Chemical additives in the juice					pH
		Citric acid g/L	Ascorbic acid g/L	Sucrose g/L	Potassium sorbate mM	Sodium benzoate mM	
A	Potable water	-	-	-	-	-	4.10-4.20
B	Sodium hypochlorite (0.25 g/L) + Potable water	-	-	-	-	-	4.10-4.20
C	Sodium hypochlorite (0.25 g/L) + potable water	2	0.3	40	-	-	3.35-3.80
D	Sodium hypochlorite				1.66	-	3.50-3.70
E	(0.25 g/L) +	2	0.3	40	3.33	-	3.55-3.75
F	potable water				6.66	-	3.45-3.65
G	Sodium hypochlorite				-	1.73	3.47-3.72
H	(0.25 g/L) +	2	0.3	40	-	3.47	3.50-3.73
I	potable water				-	6.94	3.50-3.60

The use of chemical preservatives is necessary to assure microbial safety in unpasteurized juice. In the present work, tested concentrations of potassium sorbate (MW = 150..22) and sodium benzoate (MW = 144.10) (1.66 to 6.94 mM, samples D through I in Table 1) were selected according to the values permitted by the Argentinian Regulations (Código Alimentario Argentino, Art. Nr. 1040, Res. 2067, 11.10.88) that determines a maximum level of 1g/L for sorbate (6.66 mM) or benzoate (6.94 mM) application.

The effect of packaging in plastic films with different oxygen permeabilities was also analyzed. Aliquots of 30 ml of the different samples (Samples A through I in Table 1) were packaged in polyethylene bags (Oxygen permeability, PO_2: 5000 $cm^3/m^2/24$ hs/atm at 23 °C). To determinate the effect of low gaseous permeability films, aliquots of 30 ml from the different juice samples were previously packaged in polyethylene (avoiding residual air in contact with the juice), and then packaged under vacuum in EVA-SARAN-EVA (ESE) film (Grace, Argentina) (PO_2: 50 $cm^3/m^2/24$ hs/atm at 23 °C). Samples of packed juice were stored at 10 °C for 35 d.

Physico-chemical determinations

pH and titratable acidity was determined by the AOAC 22058 method (AOAC 1984). Samples were prepared according to the AOAC 22008 method for fresh fruits (AOAC 1984); titratable acidity was expressed as meq of citric acid/100ml of juice. Concentration of organic acids in the juice were determined by HPLC (Waters, Milford, Mass., U.S.A.) 996 photodiode array detector, 717 plus autosample and water 600 pump) using Aminex HPX-87H-Biorad column, H_2SO_4 0,009 N as mobile phase. Absorbance spectrum was analyzed at 214 nm for citric and malic acids and at 245 nm for ascorbic acid. Total and reducing sugars were determined spectrophotometrically by a modified Somogyi-Nelson method (Southgate 1976). Non reducing sugars were calculated by difference between total and reducing sugars. Soluble solids were determined at 20 °C refractometry using Abbe 60 refractometer; total solids were determined in vacuum at 60 °C. Insoluble solids were calculated as the difference between total and soluble solids.

Isolation and identification of predominant microorganisms

Isolation of microorganisms was performed in Yeast Glucose Chloramphenicol (YGC) and Plate Count Agar (PCA). Gram and methylene blue coloration was applied; light microscopy was used for microscopic observation.

Isolated yeasts were identified according to Kurtzman and Fell (1998). Molds were identified according to their macroscopic and microscopic characteristics, after growth on YGC.

The presence of lactic acid bacteria (LAB) in orange juice packaged in polyethylene and ESE, stored at 10 °C and 30 °C was studied in OSA medium (Oxoid) (30 °C, 48 hr) (Parish and Higgins 1988) followed by Gram coloration of the isolated colonies.

Microbial analysis

At different storage times, a 20 ml sample of orange juice from each assayed condition (Table 1) was placed into a Stomacher bag with 80 ml of peptone water 0.1% and was homogenized for 60 sec. Serial dilutions were plated onto duplicate plates of Plate Count Agar (PCA) for total microbial counts (48 hr at 30 °C) and psychrotrophic microorganisms (7 d at 4 °C); Yeast Glucose Chloramphenicol Agar (YGC) for molds and yeast counts (5 d at 30 °C) and Orange Serum Agar (OSA, Oxoid) for tolerant acid microbial counts (5 d at 30 °C).

The final quality of the product was tested using the most probable number (MPN) method for coliform counts. For the samples packaged in ESE film the sulfite-reducing *Clostridium* counts were done into SPS agar (sulfadiazine polymyxin sulfite) by MPN method and incubated in anaerobiosis.

Storage life

Storage life was defined as the time to reach microbial counts of 10^6 CFU/ml (Howard and Dewi 1995). Confidence limits of the storage life were calculated considering the average experimental error in the microbial counts.

Modeling of microbial growth

Mathematical models allow to analyze the effect of different factors on microbial growth parameters. One of the recommended models (Gibson and others 1988; Zwietering and others 1990) is the modified Gompertz equation whose expression is $\log N = a + c.\ exp\ (-exp\ (-b\ (t-m)))$, where $\log N$: decimal logarithm of microbial counts [log(CFU/ml)] at time t; a: log N_o asymptotic log count as time decreases indefinitely [log(CFU/ml)]; c: number of log cycles of growth [log(CFU/ml)]; m: time required to reach the maximum

growth rate [d]; *b*: relative growth rate at time m [1/d].

From these parameters, the exponential growth rate (μ = b.c/e) [log(CFU/ml)/d], with e = 2.7182), lag phase duration ((LPD = m – (1/b) + ($\log N_o$ – a)/(b.c/e) [d] (McMeekin and others 1993)) and maximum population density (MPD = a + c [log(CFU/ml)]) were derived. Gompertz equation was applied to every culture in which microbial growth was detected. The equation was fitted to growth data using nonlinear regression modulus of the SYSTAT software (SYSTAT, Evanston, IL, U.S.A). The selected algorithm calculated the set of parameters with the lowest residual sum of squares (RSS) and 95% confidence interval.

When preservatives produced bactericidal effect, a linear model was applied: $\log N = \log N_0 + R\ (t - LPD)$, where R is the decline rate (log(CFU/ml)/d) and adopted negative values.

Statistical analysis

Analysis of variance (ANOVA) and the test of mean comparisons according to Fisher LSD, were applied to analyse data; levels of significance were 0.05 and 0.01. The statistical computer system package (SYSTAT Inc., (Evanston, Ill., U.S.A.) 1990 version 5.0) was used in all cases.

Results and Discussion

Physico-chemical characterization of the juice

PH values of the analyzed orange juices ranged between 4.10 and 4.19; titratable acidity between 6.07 to 6.10 meq/100ml. HPLC determinations gave the following content ranges: citric acid 878 to 890 mg/100ml, malic acid 333 to 340 mg/100ml and ascorbic acid 35 to 40 mg/100ml. Average reducing sugars, 2.43 g/100ml, total sugars, 7.49 g/100ml, nonreducing sugars, 5.06 g/100ml; soluble solids at 20 °C, 9.20 °Brix, total solids, 9.43 g/100ml, insoluble solids, 0.23 g/100ml.

Isolation and identification of predominant microorganisms

Predominant microorganisms growing in natural unpasteurized orange juice with and without additives were molds and yeasts. Identified yeasts were *Rhodotorula rubra* and *Candida quercitrusa*, in agreement with the reports of Brackett (1984). The isolated mold colonies were identified according to their characteristic morphology as: *Cladosporium* sp., *Penicillium* sp., *Epicoccum* sp., *Stemphylium* sp. and *Drechslera* sp.

According to Parish and Higgins (1989) fungi isolated from unpasteurized orange juice were: *Candida maltosa, C. sake, Fusarium* sp., *Geotricum* sp., *Hanseniaspora* sp., *H. guilliermondii, Penicillium* sp., *Pichia membranaefaciens, Sacharomyces cerevisiae, Schwanniomyces occidentalis,* and *Torulaspora delbrueckii.*

Microbial counts obtained in the different media (PCA, YGC and OSA) did not show significant differences; molds and yeasts were observed in all cases.

Although Parish and Higgins (1988) reported the presence of *Lactobacillus brevis, L. plantarum, L. thermophilus, Leuconostoc mesenteroides* and *Le. dextranicum* in citrus products, in our work, lactic acid bacteria were not isolated from juices stored at 10 °C or at 30 °C in both packaging films.

Effect of the peel washing procedure and the addition of natural preservatives on microbial flora in orange juice

Initial total microbial counts in juices obtained from oranges whose peel was washed with water (Sample A) or with 0.25 g/L sodium hypochlorite (Sample B) ranged between 10^1 - 10^2 CFU/ml, being these values lower than those reported by Parish (1998) and similar to those obtained by Petrel and others (1998) for peeled orange portions. Figure 2 shows the effect of the peel washing procedure and the addition of natural preservatives (citric and ascorbic acid) on microbial growth in orange juice. In this figure, mold and yeast counts in juice samples without additives (samples A and B) and in sample C containing 2 g/L citric acid, 0.3 g/L ascorbic acid and 40 g/L sucrose are observed during storage at 10 °C.

The effect of the packaging film (polyethylene with high oxygen permeability and EVA-SARAN-EVA with low gaseous permeability) can also be analyzed from Figure 2. Microbial counts were modeled using Gompertz equation and the full lines represent the fitted curves.

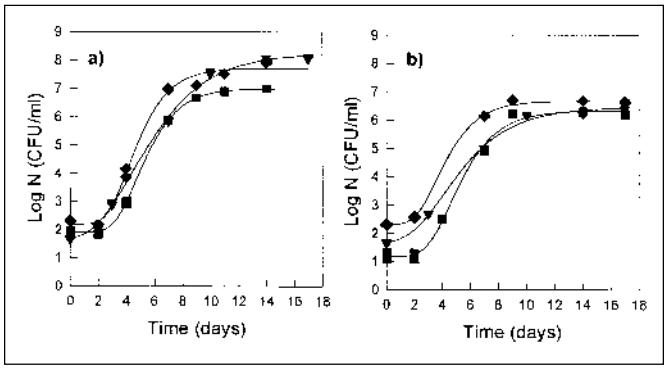

Figure 1—Experimental data and Gompertz model fitted curves showing the growth of molds and yeasts in: ◆ sample A (juice from oranges whose peel was washed with water), ■ sample B (juice from oranges whose peel was washed with sodium hypochlorite 0.25 g/L), ▼ sample C (juice from oranges whose peel was washed with sodium hypochlorite 0.25 g/L, and contains 0.3 g/L ascorbic acid 2 g/L citric acid, and 40 g/L sucrose). (a) polyethylene packaging (b) ESE film packaging. Storage temperature: 10 °C.

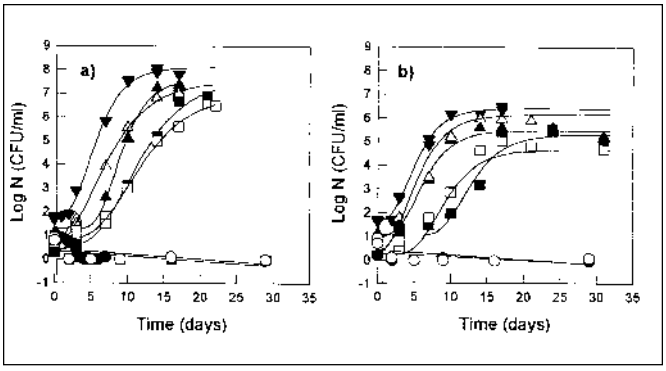

Figure 2—Microbial counts showing the effect of sorbate and benzoate on molds and yeasts in orange juice packaged in (a) polyethylene (b) ESE film. ▼ sample C, ▲ sample D (with 1.66 mM potassium sorbate), △ sample G (with 1.73 mM sodium benzoate), ■ sample E (with 3.33 mM potassium sorbate), □ sample H (with 3.47 mM potassium benzoate), ● sample F (with 6.66 mM potassium sorbate), ○ sample I (with 6.94 mM sodium benzoate). Storage temperature 10 °C. Solid lines correspond to the fitting of Gompertz or linear models.

Table 2—Mathematical modelling of molds and yeast growing in orange juice samples, packaged in polyethylene or ESE and stored at 10 °C. Gompertz parameters (a, b, c, m), derived parameters (μ, lag phase duration (LPD), maximum population density (MPD= a+c) and slopes (R) of the linear model. Key to samples composition in Table 1.

Sample	Packaging film	Gompertz and derived parameters							Linear model
		a log(CFU/ml)	b 1/d	c log(CFU/ml)	m d	μ log (CFU/ml)/d	LPD d	MPD log(CFU/ml)	R log(CFU/ml)/d
A	Polyethylene	2.19±0.02	0.65±0.01	5.52±0.34	4.18±0.13	1.32±0.25	2.64±0.43	7.71±0.60	—
B		1.92±0.03	0.62±0.03	5.09±0.06	4.76±0.05	1.16±0.33	3.15±0.50	7.01±0.30	—
C		1.63±0.01	0.35±0.02	6.61±0.15	4.49±0.15	0.85±0.35	1.63±1.22	8.24±0.40	—
D		1.28±0.14	0.40±0.07	6.34±0.29	8.21±0.26	0.93±0.62	5.71±1.73	7.62±0.66	—
E		0.56±0.14	0.23±0.03	7.04±0.42	9.91±0.36	0.60±0.45	5.56±3.33	7.60±0.75	—
F		—	—	—	—	—	—	—	–0.02±0.01
G		0.28±0.18	0.24±0.02	7.24±0.28	5.29±0.24	0.64±0.38	1.12±2.50	7.52±0.68	—
H		0.83±0.11	0.22±0.02	6.15±0.27	10.08±0.31	0.50±0.32	5.53±2.97	6.98±0.62	—
I		—	—	—	—	—	—	—	–0.02±0.01
A	ESE	2.30±0.06	0.63±0.07	4.37±0.08	3.61±0.17	1.01±0.43	2.02±0.78	6.67±0.37	—
B		1.17±0.12	0.56±0.07	5.16±0.19	4.61±0.21	1.06±0.51	2.82±0.96	6.33±0.56	—
C		1.64±0.09	0.39±0.03	4.81±0.13	4.15±0.17	0.69±0.31	1.59±1.21	6.45±0.47	—
D		1.04±0.19	0.39±0.06	4.44±0.23	5.34±0.47	0.64±0.41	2.78±1.75	5.48±0.65	—
E		1.05±0.19	0.33±0.09	4.28±0.31	0.12±0.76	0.52±0.48	8.67±2.89	5.33±0.71	—
F		—	—	—	—	—	—	—	–0.01±0.01
G		0.20±0.74	0.31±0.08	5.96±0.89	4.09±0.97	0.68±0.63	0.86±3.10	6.16±1.28	—
H		0.53±0.19	0.33±0.08	4.45±0.30	8.13±0.51	0.54±0.47	5.10±2.69	4.98±0.70	—
I		—	—	—	—	—	—	—	—0.02±0.01

SK: potassium,sorbate, BZ: sodium benzoate

Table 3—Inhibition Indices for the different tested samples in both packaging film (Key to sample notation in Table 1)

	Inhibition Index	
	Packaging film	
Sample	Polyethylene	ESE
D	0	0
E	0.2	0.8
F	1.0	1.1
G	0	0.2
H	0.3	0.4
I	1.0	1.1

Natural juice from oranges washed with hypochlorite (Sample B) showed microbial counts similar to those corresponding to fruits only washed with water (Sample A). Table 2 shows the Gompertz and derived parameters obtained from the different samples; nonsignificant differences in the derived parameters were observed ($p > 0.05$).

The effect of film permeability was evidenced by a decrease of the maximum population density (MPD) in samples A and B packaged in ESE film (MPD between 6.33 and 6.67 log(CFU/ml)) with reference to the same samples packaged in polyethylene (MPD between 7.01 and 7.71 log(CFU/ml)) (Table 2). Addition of 2 g/L citric acid and 0.3 g/L ascorbic acid (sample C) to natural untreated orange juice (sample B) markedly decreases μ values of mold and yeasts (Table 2) in both packaging films.

Effect of potassium sorbate and sodium benzoate on microbial flora in orange juice

The effect on microbial flora of different concentrations of potassium sorbate and sodium benzoate added to sample C (juice containing only natural preservatives) as a function of storage time at 10 °C is shown in Figure 2; Gompertz and linear models (solid lines) were fitted to the experimental data. Gompertz and derived parameters are shown in Table 2; sample D shows that the addition of 1.66 mM potassium sorbate to sample C produced a marked increase in the lag phase duration, mainly in polyethylene packaged samples. The use of similar levels of benzoate (sample G) did not produce significant changes neither in μ nor in LPD values. Higher preservative concentrations (3.33 mM sorbate or 3.47 mM benzoate, samples E and H respectively) increased lag phase duration and decreased μ in both packaging films. MPD values, in polyethylene packaging were similar to the sample C, however in ESE film a decrease in MPD was observed.

The reduction of the maximum population density was more important in ESE packaging film having low gas permeability. The maximum applied concentrations (6.66 mM of sorbate in sample F or 6.94 mM benzoate in sample I) produced a slightly bactericidal effect; in this case a linear model was applied and the slope R adopted negative values that were near zero.

As observed, maximum population density (MPD) decreased significantly when the preservative concentration increased. The use of low gaseous permeability films improved the performance of the preservatives, obtaining lower MPD values, in comparison to samples packaged in polyethylene.

Inhibition Index

In order to analyze the inhibitory action of potassium sorbate and sodium benzoate, an Inhibition Index (II) was defined as follows: $II = 1 - (\log(N/N_0)_{treated} / \log(N/N_0)_{control})$, where N = number of microorganisms at time t, No = initial level of microorganisms. The values of $\log(N/No)_{treated}$ and $\log(N/No)_{control}$ were evaluated at the time the sample C reached the stationary phase (14 and 17 d for the samples packaged in polyethylene and ESE, respectively). It must be noted that, if the Inhibitory Index is equal to 1, then the microorganisms in the treated samples remain into lag phase (N = No); should the Index surpassed 1, a bactericidal action takes place and $\log(N/No)_{treated}$ has negative values while, II = 0, indicates a microbial growth similar to that of control samples. Moreover, II values between 0 and 1 reflect a definite microbial growth at a lower rate than that of control samples because of the preservative action. Inhibition Indices were calculated for each assayed condition.

Table 4—Storage life of the different orange juice samples stored at 10 °C. (Key to sample notation in Table 1)

	Storage life (days to reach $10^{\pm 0.2}$ CFU/ml)	
	Packaging film	
Sample	Polyethylene	ESE
A	5.7 ± 0.2	6.7 ± 0.7
B	7.4 ± 0.4	9.7 ± 1.4
C	7.5 ± 0.5	10.4 ± 1.5
D	11.5 ± 0.5	> 20
E	15.7 ± 0.7	> 20
F	> 20	> 20
G	11.5 ± 0.5	14.0 ± 2.2
H	19.5 ± 2.7	> 20
I	> 20	> 20

Inhibition index values were obtained from experimental data and are shown in Table 3. Concentrations of 1.66 mM potassium sorbate or 1.73 mM sodium benzoate (sample D and G) had no inhibitory effect on mold and yeasts. The inhibitory indices increased when 3.33 mM potassium sorbate or 3.47 mM sodium benzoate were added (sample E and H); this effect was more significant in ESE film than in polyethylene. The addition of 6.66 mM sorbate (sample F) or 6.94 mM benzoate (sample I) produced a slightly bactericidal effect on the microbial flora on both packaging films that is characterized by an inhibition index > 1 (Table 3).

Storage life

Confidence limits of the storage life were determined, considering that the average experimental error in the microbial counts was 0.2 log CFU/ml.

Storage life values (time to reach microbial counts of $10^{6 \pm 0.2}$ CFU/ml) at 10 °C for the different samples and packaging conditions are shown in Table 4. Juices obtained from oranges whose peel was washed with water (sample A) showed a mean storage life value of 5.7 d when polyethylene packaging was used; in ESE film, storage life was 6.7 d (Table 4). Washing the orange peel with a solution of sodium hypochlorite (sample B) and using simultaneously films of low gas permeability allowed to extend the storage life of the juice from 7.4 to 9.7 d. The addition of natural preservatives to the juice (sample C) has not a significant effect on storage life values in comparison to sample B in both packaging films.

Storage life values ranging between 11.5 and 15.7 d, were obtained for samples D and E packaged in polyethylene and treated with potassium sorbate in concentrations of 1.66 and 3.33 mM, respectively. For vacuum packaging in ESE film, the storage life reached values > 20 d for both samples. The use of similar sodium benzoate levels (samples G - H) allowed to reach storage life values of 11.5 and 19.5 d in polyethylene. With the low gaseous permeability film (ESE), storage life was extended to 14 and > 20 d for G and H samples, respectively. Shelf life values > 20 d were obtained with 6.66 mM of potassium sorbate (sample F) or 6.94 mM sodium benzoate (sample I) , for both packaging films.

The combined effect of chemical preservatives (potassium sorbate and sodium benzoate) and low gaseous permeability film allowed to extend storage life of unpasteurized orange juice.

The evaluation of the final quality of the juice in all the assayed conditions (sample A - I) packaged in both plastic films showed values lower than 2 MPN/ml for Total Coliforms and sulfite-reducing Clostridium, indicating the good sanitary conditions of the product.

References

Andrés SC, Giannuzzi L, Zaritzky NE. 1999. Effect of chemical preservatives and packaging film permeability on microbial growth in refrigerated orange juice. In: Proceeding of the Seventeenth International Conference of the International Committee on Food Microbiology and Hygiene (ICFMH), Tuijtelaars ACJ, Samson RA, Rombouts FM, Notermans S, editors. Sept. 1999. Veldhoven, Holland, p. 212-217.

AOAC. 1984. *Official Methods of Analysis*, 14th ed. Association of Official Analytical Chemists, Washington, DC.

Ayhan Z, Chism GW, Richter ER. 1998. The shelf-life of minimally processed fresh cut melons. J. Food Qual. 21: 29-40.

Brackett RE. 1984. Microbiological Spoilage and Pathogens in Minimally Processed Refrigerated Fruits and Vegetables. In: *Minimally Processed Refrigerated Fruits & Vegetables*, R.C. Wiley, editor. New York: Chapman & Hall.p. 269-312.

Código Alimentario Argentino. 1996. De La Canale y Asociados.

Deak T, Beuchat LR. 1993. Yeast Associated with Fruit Juices Concentrates. J. Food Prot. 56: 777-782.

Gibson A M, Bratchell N, Roberts TA. 1988. Predicting microbial growth: growth response of salmonellae in a laboratory medium as affected by pH, sodium chloride and storage temperature. Int. J. Appl. Microbiol. 6: 155-178.

Howard LR, Dewi T. 1995. Sensory, microbiologycal and chemical quality of minni-peeled carrots as affected by edible coating treatment. J. Food Sci. 60: 142-144.

Kopelman IJ, Rauchwerger M. 1984. Shelf life and microbial growth kinetic of fresh citrus juices: Shamouti orange. J. Food Proc. Preserv. 8: 241-250.

Kurtsman CP, Fell JW. 1998. *The Yeast, a Taxonomic Study*. Fourth Edition. Amsterdam. Elsevier Science. p. 543, 820-822.

Matthys A. 1999. Jugos de frutas y vegetales en los Estados Unidos. La Alimentación Latinoamericana 229: 56-59.

McMeekin TA, Olley J, Ross T, Ratkowsky DA. 1993. Predictive Microbiology: Theory and Application. New York. John Wiley and Sons. p 35.

Pao S, Petracek PD. 1997. Shelf life extension of peeled oranges by citric acid treatment. Food Microbiol. 14: 485-491.

Parish ME. 1991. Microbiological concerns in citrus juice processing. Food Techn. 45: 128-133.

Parish ME. 1998. Orange juice quality after treatment by thermal pasteurization or isostatic high pressure. Lebensm.-Wiss.u.-Technol. 31: 439-442.

Parish ME, Higgins D. 1988. Isolation and identification of lactic acid bacteria from samples of citrus molasses and unpasteurized orange juice. J. Food Sci. 53 (2): 645-646.

Parish ME, Higgins D. 1989. Yeasts and molds isolated from spoiling citrus products and by-products. J. Food Prot. 52: 261-263.

Petrel MT, Fernández PS, Romojaro F, Martínez A. 1998. The effect of modified atmosphere packaging on ready-to-eat oranges. Lebensm.-Wiss.u.-Technol. 31: 322-328.

Ross T, McMeekin TA. 1991. Predictive microbiology application of square-root model. Food Aust. 43: 202-207.

Southgate DAT. 1976. *Determination of Food Carbohydrates*. London. Applied Science, p.105-106.

Zwietering MH, Jongenburger FM, Roumbouts M, Van´t Riet K. 1990. Modelling of the bacterial growth curve. Appl. Environ. Microbiol. 56: 1875-1881

MS 20000418

The financial support provided by CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas, Project 0296/98), Projects BID 802 OC-AR PID Nro. 358 and PICT98 0904579 from Agencia Nacional de Promoción Científica y Tecnológica, and University of La Plata, Argentina, is gratefully acknowledged.

Authors Andrés, Giannuzzi, and Zaritzky are with the Centro de Investigación y Desarrollo en Criotecnología de Alimentos Address inquiries to author Zaritzky. (E-mail: zaritzky@volta.ing.unlp.edu.ar)