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Quality Parameters of Packaged Refrigerated Apple Cubes in Orange Juice

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The quality of apple cubes (Red Delicious and Granny Smith) maintained in pasteurised or fresh orange juice with sucrose and chemical preservatives: acidulants (citric and ascorbic acids) and potassium sorbate or sodium benzoate, packaged in plastic films of different gaseous permeability (polyethylene (PE) and EVA-SARAN-EVA (ESE)), was analysed during storage at 10 °C. Surface colour, microbial growth and instrumental texture changes were measured. Microbial flora was identified, and its growth was mathematically modelled. Shelf-life values of 10 d in polyethylene and higher than 25 d in ESE film, maintaining acceptable quality attributes, were obtained for both apple varieties in pasteurised juices. For Red Delicious in unpasteurised orange juice with acidulants and potassium sorbate (0.125–0.250 g/kg), shelf-life values were 7.5–8.5 d in PE and higher than 15 d in ESE film. For Granny Smith apples in unpasteurised juice without potassium sorbate, shelf-life values were 10 d in PE and higher than 15 d in ESE. Higher concentrations (0.375–0.50 g/kg) of potassium sorbate adversely affected colour. Sodium benzoate was discarded as preservative because it produced severe browning in both apple varieties. All the samples showed safe sanitary conditions; coliforms microorganisms did not grow in any of the tested conditions.

Keywords: refrigerated apple cubes; film packaging; orange juice; preservatives

Introduction

'Ready-to-eat' salad fruits are normally prepared with fresh fruits in low pH juices such as orange juice. Cut apples are usually included in these salad fruits, even though apple is a typical fruit susceptible to enzymatic browning (Kim *et al.*, 1993). Processing, although minimally, may increase the microbial spoilage of the fruit through the transfer of skin microflora to flesh, where microorganisms can grow rapidly upon exposure to nutrient juices. The low pH of most fruits restricts the microflora to acid-tolerant microorganisms, such as fungi and lactic acid bacteria (LAB). The National Food Processors Association established that juices as fruit products, or as ingredients, have to be pasteurised, or must receive an equivalent process to ensure the production of a juice free of pathogenic microorganisms. The risks that accompany the consumption of nonpasteurised products require the use of chemical preservatives such as sorbate or benzoate to inhibit microbial growth. Extensive research related to preserving the quality of fresh cut fruit has been done with different apple varieties (Rocha *et al.*, 1998). Colour changes are retarded by low storage temperature, low levels of oxygen, and the addition of antioxidants. These treatments have been compared to sulphite-containing and sulphite-free treatments to preserve overall quality, especially colour. While sulphiting agents have been the standard chemical preservatives for inhibiting browning reactions, concerns over possible sensitive consumer allergic reactions have led to the use of alternatives (Monsalve-Gonzalez *et al.*, 1993). Natural acids such as citric, malic, phosphoric and ascorbic acids are used to control enzymatic browning. Besides, the use of films with low gaseous permeability allows to prolong the storage life of vegetable products; due to the lower oxygen content inside the package (Wiley, 1994).

The objectives of the present work were: (1) to analyse the quality (instrumental colour and texture modifications) and microbial growth parameters of cut

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apples (two varieties: Red Delicious and Granny Smith) maintained in pasteurised or fresh (unpasteurised) orange juice, with the addition of acidulants (citric and ascorbic acids) and also potassium sorbate or sodium benzoate, during storage at 10 °C in plastic films with different gaseous permeability; (2) to model microbial growth in the formulated systems during refrigerated storage; (3) to determine the shelf-life of the different product formulations at 10 °C.

Materials and Methods

Apples of two varieties: Red Delicious (RD) and Granny Smith (GS), Valencia oranges, and pasteurised orange juice (Cepita Peñaflor S.A., Argentina) from two consecutive years (1998–1999, March–December) were purchased in a local market and were stored at 10 °C for 24 h before performing the tests. The reagents used ascorbic and citric acids (Cicarelli, Argentina), sodium bisulphite (Mallinckrodt, U.S.A.), potassium sorbate and sodium benzoate (Inmobal, Argentina) were of analytical grade; sucrose (Ledesma, Argentina) was used as sweetener. To obtain fresh orange juice (unpasteurised), instruments, equipment and oranges were washed and brushed manually with sodium hypochlorite solution (0.25 g/kg), and finally with tap water according to previous studies (Andrés *et al*., 1999). Apples were washed with tap water, manually peeled and cut in cubes of 1 cm side. Samples contained 30 g of apple cubes in contact with 30 mL orange juice either pasteurised or fresh, with different chemical preservatives. Samples were packaged in polyethylene bags (PE) (oxygen permeability, PO_2: 5000 $cm^3/(m^2.d.atm)$ 23 °C). To determine the effect of low gaseous permeability films, half of the samples were also packaged under vacuum in EVA-SARAN-EVA (ESE) film (Grace, Argentina) (PO_2: 50 $cm^3/(m^2.d.atm)$ 23 °C). This procedure was used for both apple varieties.

Sensory analysis previously done in our laboratory using RD and GS apple varieties allowed us to determine appropriate levels of different preservatives (Andrés, 2001). Apples of both varieties were dipped in pasteurised orange juice (i) without any additive or with the addition of: (ii) citric acid (1 g/kg); (iii) a combination of ascorbic (0.150 g/kg) and citric (1 g/kg) acids; (iv) ascorbic acid (0.015 g/kg) and (v) sodium bisulphite (0.025 g/kg). Samples were packaged in both films and stored at 10 °C. Results of the sensory panel showed that, for RD apple cubes in pasteurised orange juice, the addition of citric acid or a combination of ascorbic and citric acids maintained an acceptable colour in comparison to sodium bisulphite, in both packaging films, and that GS apples also maintained an acceptable colour in both films without the addition of acidulants to the orange juice (Andrés, 2001).

Based on these results, different formulated orange juices were tested in the present work (**Table 1**). Apple cubes (RD and GS) were maintained in pasteurised (P) or fresh (N) orange juice with the addition of citric or ascorbic acids at the levels previously determined according to the sensory analysis; sodium bisulphite in pasteurised juice was used as a reference sample. Appropriate levels of sucrose were also determined as being 20 and 40 g/kg for RD and GS, respectively. In the case of unpasteurised juice the addition of sodium benzoate or potassium sorbate was also analysed (**Table 1**). Notations P1–P5 were used for pasteurised orange juices and N1–N18 for unpasteurised orange juices. The tested concentrations of potassium sorbate and sodium benzoate (0.125–0.5 g/kg product, **Table 1**) were selected according to the values allowed by the Argentinean Regulations (Código Alimentario Argentino, 1996) which determine a maximum of 1 g/kg of product for sorbate or benzoate application. Samples were stored at 10 °C for a total period of 32 d; microbial growth and instrumental measurements of surface colour and texture were evaluated over storage time.

Experimental design

(a) Apples in pasteurised juice: two varieties (RD and GS) × two packaging films (PE and ESE) × five formulated juices (P1–P5) × six storage times.
(b) RD variety in unpasteurised juice: two packaging films (PE and ESE) × nine formulated juices (N1–N9) × six storage times.
(c) GS variety in unpasteurised juice: two packaging films (PE and ESE) × nine formulated juices (N10–N18) × six storage times.

Physico-chemical determinations

Samples were prepared according to the AOAC 22008 method for fresh fruits (AOAC, 1984); pH and titratable acidity were determined by the AOAC 22058 method (AOAC, 1984); titratable acidity was expressed as meq of citric acid per kg of sample. The concentration of organic acids was determined by HPLC (Waters 996 photodiode array detector, 717 plus autosample and water 600 pump) using AMINEX HPX-87H-BIORAD column and 0.018 mol/L H_2SO_4 as the mobile phase. The absorbance spectra were analysed 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). Nonreducing sugars were calculated as the difference between total and reducing sugars. Soluble solids were determined at 20 °C by refractometry using an Abbe 60 refractometer; total solids were determined in a vacuum oven at 60 °C. Insoluble solids were calculated as the difference between total and soluble solids. All the analyses were carried out in duplicate.

Colour measurements

Surface colour was measured with a tristimulus reflectance colorimeter (Minolta CR-300, Minolta Corp., Ramsey, NJ, U.S.A.) on 16 peeled apple cubes of 1.5 cm side. Colour was recorded using the CIE-L*a*b* scale. Parameters were expressed as Δa*, Δb* and ΔL*

Table 1 Composition of the tested systems: orange juice (P: pasteurised, N: fresh unpasteurised) and apple varieties (Red Delicious (RD) and Granny Smith (GS)). Concentration of additives are expressed on final product (apple + juice) basis

		Chemical additives in the product (apple + juice)					
Apple variety	Juice	Sodium bisulphite (g/kg)	Citric acid (g/kg)	Ascorbic acid (g/kg)	Potassium sorbate (g/kg)	Sodium benzoate (g/kg)	pH[a]
Red Delicious[b]	P1	—	—	—	—	—	4.01 (0.04)
or Granny Smith[c]	P2	—	—	0.150	—	—	3.99 (0.04)
	P3	—	1	0.150	—	—	4.12 (0.01)
	P4	—	1	—	—	—	4.11 (0.01)
	P5	0.025	—	—	—	—	4.09 (0.01)
Red Delicious[b]	N1	—	1	0.150	—	—	3.57 (0.11)
	N2	—	1	0.150	0.125	—	3.62 (0.06)
	N3	—	1	0.150	0.250	—	3.70 (0.07)
	N4	—	1	0.150	0.375	—	3.60 (0.10)
	N5	—	1	0.150	0.500	—	3.55 (0.05)
	N6	—	1	0.150	—	0.125	3.61 (0.07)
	N7	—	1	0.150	—	0.250	3.65 (0.07)
	N8	—	1	0.150	—	0.375	3.70 (0.06)
	N9	—	1	0.150	—	0.500	3.55 (0.02)
Granny Smith[c]	N10	—	—	—	—	—	4.06 (0.04)
	N11	—	—	—	0.125	—	3.35 (0.07)
	N12	—	—	—	0.250	—	3.50 (0.05)
	N13	—	—	—	0.375	—	3.50 (0.12)
	N14	—	—	—	0.500	—	3.60 (0.05)
	N15	—	—	—	—	0.125	3.60 (0.05)
	N16	—	—	—	—	0.250	3.62 (0.04)
	N17	—	—	—	—	0.375	3.70 (0.05)
	N18	—	—	—	—	0.500	4.05 (0.02)

[a]Mean values and standard deviations.
[b]For RD apples, formulations include 20 g sucrose per kg product.
[c]For GS apples, formulations include 40 g sucrose per kg product.

referred to the initial values measured on cubes immersed in the corresponding juices and maintained for 2 h at 10 °C; the colour of untreated apple cubes, without juice (control) was also measured. All the measurements were carried out in quadruplicate.

Microbial growth

Natural microbial flora was analysed during refrigerated storage in duplicated samples of: (i) pasteurised orange juice (P1), (ii) fresh orange juice (N10), (iii) apple cubes of both varieties without juice, and (iv) apple cubes of both varieties with formulated juices (P1–P5 and N1–N18). At different storage times, 20 g of sample was homogenised with 80 mL 1 g/L peptone in a Stomacher for 60 s. Serial dilutions were plated on plate count agar (PCA) for total microbial counts (48 h, 30 °C) and psychrotrophic microorganism counts (7 d, 4 °C); yeast glucose cloranfenicol agar (YGC) (Merck) for mould and yeast counts (5 d, 30 °C) and orange serum agar (OSA) (Oxoid) for acid- tolerant microbial counts (5 d, 30 °C) (Parish, 1998). Microbial counts were performed in duplicate. Gram and methylene blue colorations were applied to the isolated colonies and microscopic observation was conducted using light microscopy (Ortholux II, Leitz, Germany). 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 sulphite-reducing Clostridium counts were carried out on SPS agar (sulphadiazine polymyxin sulphite) by MPN method incubating in anaerobiosis.

Modelling of microbial growth

The effect of the different formulations and packaging films on microbial growth parameters was analysed using the modified Gompertz equation (Gibson *et al.*, 1988; Zwietering *et al.*, 1990):

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

where $\log N$ is the decimal logarithm of microbial counts [log(cfu/mL)] at time t; a the $\log N_0$ is the asymptotic log count as time decreases indefinitely [log(cfu/mL)]; c the log count increment as time increases indefinitely [log(cfu/mL)]; m the time required to reach the maximum growth rate (d); and b the relative growth rate at time m (d^{-1}). From these parameters, the exponential growth rate ($\mu = bc/e$) [log(cfu/mL)/d], with $e = 2.7182$, lag phase duration ($LPD = m-(1/b)$)[d] and maximum population density ($MPD = a + c$ [log(cfu/mL)]) were derived. Gompertz's equation was applied to every culture in which microbial growth was detected. The equation was fitted to growth data using the 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 a bactericidal effect, a linear model was applied: $\log N = \log N_0 + R(t - \mathrm{LPD})$, where R is the decline rate [(log(cfu/mL)/d)] and adopted negative values.

Firmness assessment

Apple resistance to compression was determined at 25 °C with an Instron Testing machine Model 1011 (Instron Corp., Canton, MA) using a 500 N cell, a compression of 35% and a crosshead speed of 10 mm/min. Measurements were performed over the storage period. The maximum compression force was measured on peeled apple tissue cylinders 1.5 cm in diameter and 2 cm high by quadruplicate. Relative softness of the treated product packaged in the plastic films was determined during storage with respect to the untreated sample (four cylinders from each apple) according to the following expression: Relative softness $= (F2 - F1)/F1$, where $F1$ is the maximum compression force in the untreated apples (N) and $F2$ the maximum compression force in the treated apples at different times (N).

Sensory analyses

Fifteen nontrained panellists evaluated the samples. Paired comparisons for colour, firmness and flavour were done. A 5-point hedonic scale was used: 0 = dislike extremely; 2 = neither like or dislike; 4 = like extremely. The product was considered unacceptable if the score was below 2.

Statistical analyses

Analysis of variance (ANOVA) and the test of mean comparisons according to Fisher least significant difference (LSD) were applied; levels of significance were 0.05 and 0.01. A statistical computer system package (SYSTAT Inc., version 5.0) was used.

Results and Discussion

Chemical characterisation of apples and juices

Table 2 shows the chemical characterisation of both apple varieties and orange juices. HPLC results indicated that GS variety had levels of citric and malic acids higher than that for RD; GS had higher titratable

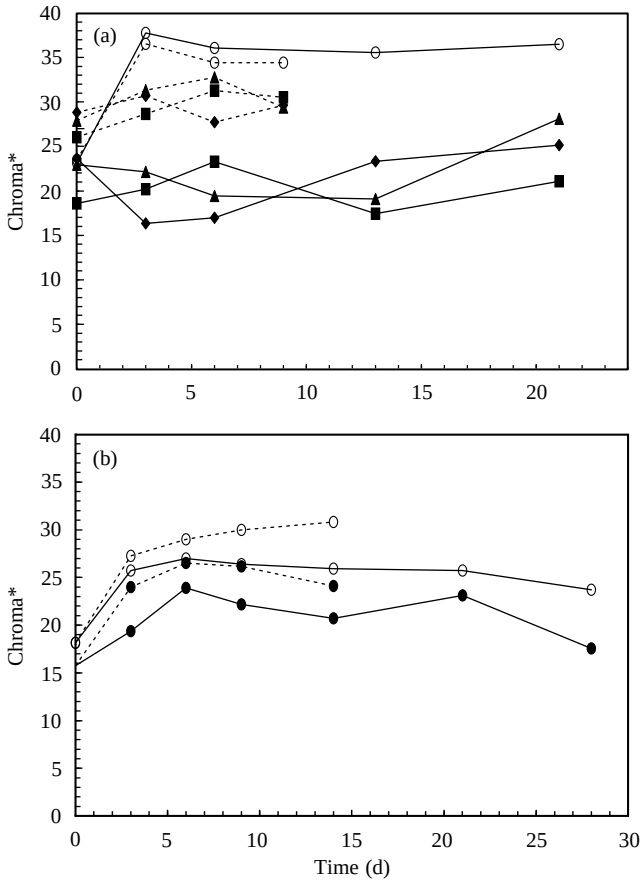

Fig. 1 Chroma* values as a function of storage time at 10 °C for apple cubes maintained in pasteurised juice formulations using different packaging films. (**a**) Red Delicious; (**b**) Granny Smith. ----, polyethylene; —— ESE film. (**a**) $LSD_{0.05} = 1.25$; (**b**) $LSD_{0.05} = 1.27$. ○, without juice; ●, P1; ■, P3; ▲, P4 ◆, P5. Keys to the formulations are in **Table 1.**

Table 2 Chemical characterisation of both apple varieties (Red Delicious and Granny Smith) and orange juices (pasteurised and unpasteurised)

	Apple varieties		Orange juice	
Determination[a]	Red Delicious	Granny Smith	Pasteurised	Unpasteurised
pH	4.05(0.01)	3.40 (0.04)	3.98 (0.03)	4.15 (0.02)
Titratable acidity (meq/kg)	33.35 (1.35)	90.50 (3.70)	111.65 (0.02)	60.85 (0.07)
Malic acid (g/kg)	4.62 (0.33)	9.56 (1.43)	4.72 (0.12)	3.32 (0.01)
Citric acid (g/kg)	0.40 (0.02)	0.59 (0.12)	9.95 (0.45)	8.75 (0.05)
Ascorbic acid (mg/kg)	1.45 (0.02)	0.05 (0.01)	155.05 (2.50)	400.08 (25.03)
Soluble solids (°Brix)	16.45 (0.25)	11.85 (0.27)	11.05 (0.02)	9.15 (0.07)
Insoluble solids (g/kg)	126.75 (0.93)	119.80 (1.80)	3.55 (0.17)	2.50 (0.02)
Total solids (g/kg)	291.25 (2.18)	238.30 (4.55)	114.05 (0.42)	94.50 (0.25)
Reducing sugars (g/kg)	34.55 (0.22)	45.55 (0.22)	52.90 (0.35)	25.10 (0.50)
Non reducing sugars (g/kg)	52.05 (0.25)	24.15 (0.52)	44.65 (0.27)	51.05 (0.22)
Total sugars (g/kg)	87.80 (0.60)	70.25 (0.57)	96.75 (0.12)	75.35 (0.22)

All reported data are the mean of duplicate determinations.
[a]Standard error within the parentheses.

acidity and lower pH as compared to RD; ascorbic acid levels for both varieties ranged between 0.05 and 1.45 mg/kg. RD showed higher levels of soluble and insoluble solids in comparison to GS. Similar results were obtained for total and nonreducing sugar content. Both orange juices had values of titratable acidity, pH and organic acid levels (citric, malic and ascorbic acids) (**Table 2**) similar to those reported by CTILF (1994), Parish (1998) and Hulme (1970). Citric acid concentration and titratable acidity were higher in the pasteurised juice; pH and ascorbic acid concentration were higher in the fresh juice.

Surface colour

The functions Chroma* $= (a^{*2} + b^{*2})^{1/2}$ and Chromaticity difference $= (\Delta a^{*2} + \Delta b^{*2})^{1/2}$ were selected because they interpreted better the visual observations made previously by a nontrained panel (**Figs 1a,b** and **2a**–**d**). The $LSD_{0.05}$ values obtained were included in the legends of the figures. Samples packaged in polyethylene bags were evaluated up to the time (i) microbial growth was evidenced by gas formation, (ii) surface growth of moulds was observed or (iii) they were rejected by the sensory panel due to browning. For RD apples, the lowest values of Chroma* corresponded to the cubes maintained in pasteurised orange juice containing (i) citric acid (P4), (ii) citric and ascorbic acid (P3), or (iii) sodium bisulphite (P5), in both PE and ESE films (**Fig. 1a**). A good colour retention was observed in these cases. Pizzocaro *et al*. (1993) found similar results when apple cubes were treated with ascorbic and citric acids reaching a 0.36 inhibition of polyphenoloxidase. According to these, it is possible to replace a concentration of 0.025 g/kg bisulphite in the product, by citric acid (1 g/kg), or a combination of ascorbic acid (0.15 g/kg) and citric acid (1 g/kg) to avoid enzymatic browning and to maintain the colour of RD apples during refrigerated storage, in both packaging films. A satisfactory colour preservation of GS in pasteurised juice without any additive (P1) for both packaging films was obtained according to Chroma* values (**Fig. 1b**). Sodium benzoate produced colour deterioration for both apple varieties in formulated orange juices (**Fig. 2a**–**d**). These results agree with those of Sapers *et al*. (1989) who reported that GS apples suffered a severe browning induced by benzoate that could be attributed to its slow conversion to a polyphenoloxidase substrate.

Due to these negative results, sodium benzoate was discarded as a preservative in our work. Potassium sorbate in levels ranging between 0.125 and 0.25 g/kg gave a good colour retention for both apple varieties;

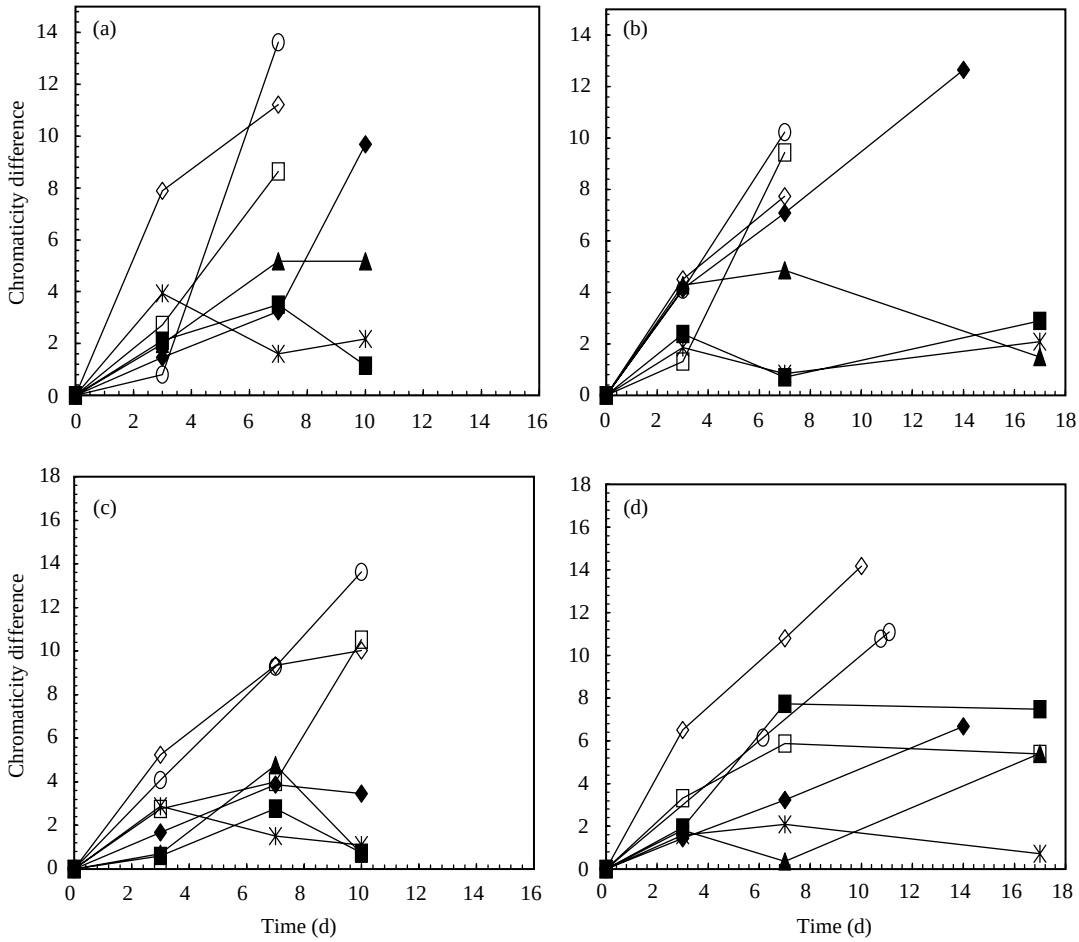

Fig. 2 Chromaticity difference values as a function of storage time at 10 °C of apple cubes maintained in fresh juice formulations using different packaging films. (**a**,**b**) Red Delicious; (**c**,**d**) Granny Smith. (**a**,**c**) polyethylene. (**b**,**d**) ESE film. (**a**) $LSD_{0.05} = 0.95$; (**b**) $LSD_{0.05} = 0.91$; (**c**) $LSD_{0.05} = 0.81$; (d) $LSD_{0.05} = 0.83$. (**a**,**b**) ⨯, N1; ▲, N2; ■, N3; ◆, N5; □, N7; ○, N8; ◇, N9. (**c**,**d**) ⨯, N10; ▲, N11; ■, N12; ◆, N14; □, N16; ○, N17; ◇, N18. Keys to the formulations are in **Table 1**.

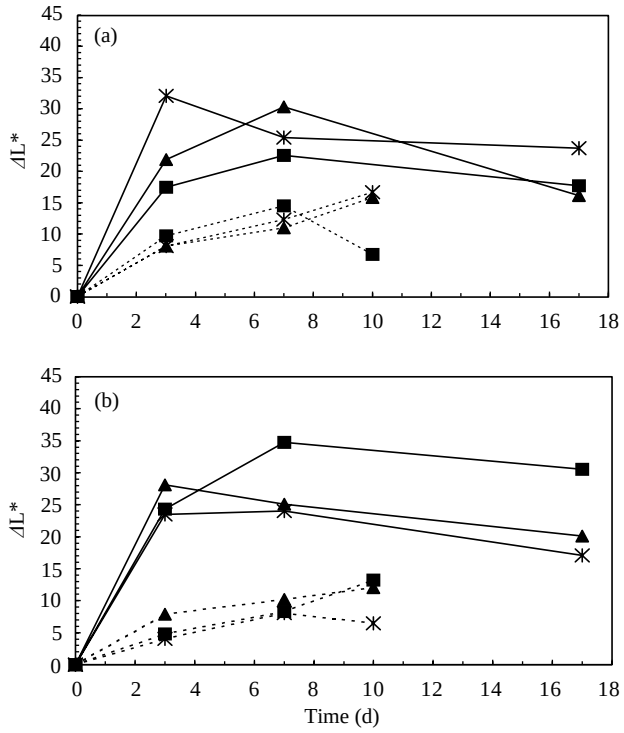

Fig. 3 ΔL* values as a function of storage time at 10 °C of apple cubes maintained in fresh juice formulations using different packaging films. (**a**) Red Delicious; (**b**) Granny Smith. ---- polyethylene; ——, ESE film. (**a**) $LSD_{0.05} = 2.26$; (**b**) $LSD_{0.05} = 2.55$. (**a**) ✳, N1; ▲, N2; ■, N3. (**b**) ✳, N10; ▲, N11; ■, N12. Keys to the formulations are in Table 1.

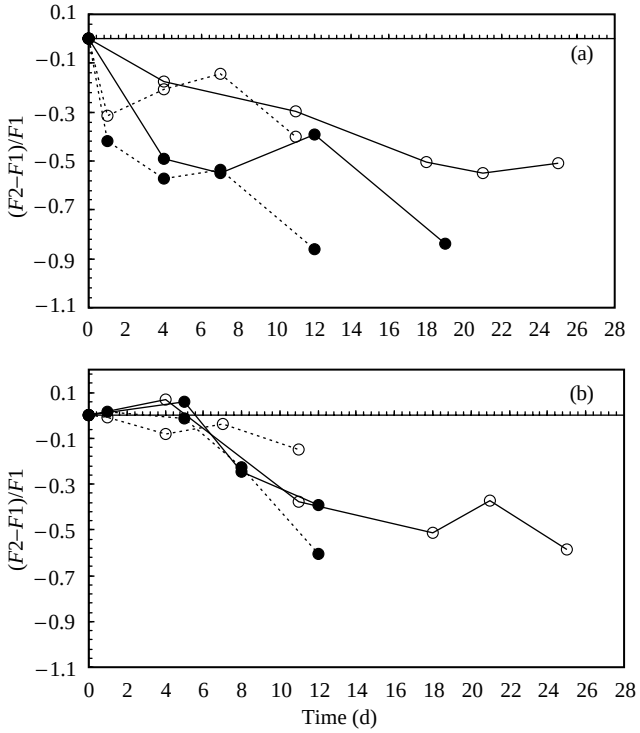

Fig. 4 Relative softness as a function of storage time at 10 °C of apple cubes (**a**) Red Delicious and (**b**) Granny Smith in different juice formulations using different packaging films: -- Polyethylene; —— ESE. (**a**) ●, P3; ○, N1. $LSD_{0.05} = 0.04$; (**b**) ●, P1; ○, N10. $LSD_{0.05} = 0.03$

however, levels of 0.375–0.50 g/kg produced severe browning. Chromaticity differences were lower for ESE than for PE packaging ($P<0.01$) for both apple varieties, showing that low oxygen permeability films led to a better colour retention. Samples packaged with ESE film were translucent or waterlogged at the end of the storage period, with high ΔL* values for both apple varieties (**Fig. 3**). Sapers (1993) described a similar effect in cut apples; however, in our assays, the apples packaged in low permeability films were not rejected by the sensory panel.

Instrumental texture

Figure 4a and **b** shows relative softness values for both varieties of apples packaged in different films during refrigerated storage. The values of $LSD_{0.05}$ are included in the legends of the figures. Differences in relative softening for both packaging films and under all treatments, were nonsignificant ($P>0.05$) up to 4 and 8 d of storage for RD and GS varieties, respectively. Film type was not a significant factor ($P>0.05$) for either apple variety. The relative softness of RD apples was significantly lower ($P<0.05$) in pasteurised orange juice with citric acid, ascorbic acid and sucrose (P3) than in fresh juice with the same additives (N1) (**Fig. 4a**). However, the type of juice did not significantly affect the texture of GS apples (**Fig. 4b**).

Microbial flora

Under all tested conditions, microbial counts in PCA (4 and 30 °C), YGC and OSA media were not significantly different; psychrotrophic moulds and yeasts were observed in all cases. These results were confirmed by light microscopy techniques. Initial microbial counts in apple cubes of both varieties ranged between 10^2 and 10^3 cfu/g; similar results were reported by Brackett (1994). Initial microbial counts in pasteurised juice were 10 cfu/mL, and in fresh juice counts ranged between 10^1 and 10^2 cfu/mL. Microbial counts in both apple varieties without orange juice, packaged in PE and stored at 10 °C, reached 10^6 cfu/g at 10 d storage. However, when ESE film was used, microbial counts were below 10^5 cfu/g for both apple varieties even after 32 d at 10 °C. In P1 juice, counts were below 10 cfu/mL for both packaging films during storage at 10 °C. The fresh juice without additives (Juice N1) reached values of 10^6 cfu/mL at 7 and 10 d storage in PE and ESE film, respectively.

Modelling of microbial growth

Gompertz's and linear models were fitted to mould and yeast counts in RD and GS apple cubes under all tested conditions (**Fig. 5a–d**). Gompertz's parameters (*a*, *b*, *c*, *m*) and the derived parameters (exponential growth rate (μ); lag phase duration (LPD), maximum population density (MPD)) and decline rates (*R*) of the linear model, are shown in **Table 3**. Potassium sorbate showed an inhibitory effect on moulds and yeasts. In PE packaging, as the concentration of potassium sorbate

Table 3 Parameters of the fitted equations to yeast and mould counts in Red Delicious and Granny Smith apple cubes, immersed in different orange juices with preservatives, packaged in PE or ESE film and stored at 10 °C

			Gompertz model							
			Parameters[a]				Derived parameters			
Apple variety	Packaging film	Juice	a [log (cfu/g)]	b (d^{-1})	c [log (cfu/g)]	m (d)	μ [log (cfu/g)/d]	LPD (d)	MPD [log (cfu/g)]	Linear model R [log (cfu/g)/d]
Red delicious	Polyethylene	P3	3.21 (0.12)	0.26 (0.05)	4.27 (0.42)	7.03 (0.45)	0.42	3.46	7.48	—
		N1	3.19 (0.26)	0.39 (0.06)	5.20 (0.34)	3.59 (0.36)	0.76	1.06	8.39	—
		N2	3.09 (0.14)	0.45 (0.08)	4.62 (0.22)	5.55 (0.35)	0.76	3.33	7.71	—
		N3	3.84 (0.08)	0.38 (<0.01)	2.99 (0.12)	4.76 (0.27)	0.42	2.13	6.83	—
		N4	—	—	—	—	—	—	—	0.01
		N5	—	—	—	—	—	—	—	−0.01
	ESE	P3	3.42 (0.17)	0.25 (0.14)	2.15 (0.58)	17.73 (2.18)	0.20	13.73	5.57	—
		N1	3.45 (0.12)	0.29 (0.05)	3.54 (0.21)	7.69 (0.45)	0.37	4.19	6.99	—
		N2	—	—	—	—	—	—	—	0.01
		N3	—	—	—	—	—	—	—	−0.05
		N5	—	—	—	—	—	—	—	−0.03
Granny Smith	Polyethylene	P1	2.35 (0.09)	0.55 (0.06)	3.86 (0.14)	4.89 (0.17)	0.78	3.07	6.21	—
		N10	2.58 (0.10)	0.38 (0.05)	4.55 (0.19)	6.24 (0.26)	0.64	3.61	7.13	—
		N11	3.20 (0.05)	0.27 (0.04)	2.17 (0.1)	10.26 (0.37)	0.22	6.56	5.37	—
		N12	—	—	—	—	—	—	—	−0.09
		N14	—	—	—	—	—	—	—	−0.02
	ESE	P1	—	—	—	—	—	—	—	0.05
		N10	2.50 (0.06)	1.14 (0.55)	2.08 (0.08)	6.99 (0.11)	0.87	6.11	4.58	—
		N11	—	—	—	—	—	—	—	0.01
		N12	—	—	—	—	—	—	—	−0.06
		N14	—	—	—	—	—	—	—	−0.03

Gompertz parameters (a, b, c, m) and derived parameters [exponential growth rate (μ); lag phase duration (LPD), maximum population density (MPD)] were obtained in the cases of microbial growth and decline rate (R) of the linear model when bacteriostatic or bactericidal affect as observed. Keys to the treatments are in Table 1.

[a]Standard error within the parentheses.

increased, the exponential growth rate (μ) decreased, LPD increased, and MPD decreased. In ESE film, levels of potassium sorbate higher than 0.125 g/kg, showed bacteriostatic or bactericidal effects (**Table 3**). With PE packaging, RD cubes with fresh orange juice containing citric and ascorbic acids, sucrose (20 g/kg), and 0.125 g potassium sorbate per kg (N2 sample) showed lower microbial counts in comparison to the juice N1 without preservatives. The addition of 0.250 g potassium sorbate per kg (N3 sample) produced lower values of the exponential microbial growth rate and of the final counts. In ESE film, the addition of potassium sorbate at concentration levels of 0.125 g/kg (N2) and 0.250 g/kg (N3) produced bacteriostatic and bactericidal effect, respectively (R values were zero or slightly negative). When RD cubes were immersed in pasteurised juice (P3) and packaged in PE, μ and MPD values were similar to those obtained with fresh juice containing 0.250 g potassium sorbate per kg (N3 sample) (**Table 3**).

For GS apples (**Fig. 5c** and **d**) in fresh juice with sucrose, the addition of 0.125 g potassium sorbate per kg (N11) in samples packaged in PE led to a lower value of MPD and a lower μ in comparison to the system containing pasteurised (P1) or fresh juice (N10) without preservatives. When 0.250 g potassium sorbate per kg (N12) was added, a bactericidal effect (negative R values) was observed. In ESE film, the addition of the lowest level of potassium sorbate (0.125 g/kg, N11) produced a bacteriostatic effect. For both apple varieties in both packaging films, concentrations of 0.50 g potassium sorbate per kg (N5 and N14) showed a slightly lethal effect on microbial flora and a decrease of the initial microbial load (Fig. 5). All the samples packaged in both films showed total coliforms and sulphite-reducing Clostridium values below 2 MPN/mL, indicating that the product had good sanitary conditions.

Sensory analyses on samples in fresh juices

Sensory evaluation of colour, firmness and flavour of both apple varieties maintained in the formulated fresh orange juices (N1–N5 for RD, and N10–N14 for GS) indicated good acceptability (score >2) for all tested conditions (**Table 4**).

Shelf-life

According to previous results, shelf-life was defined as the number of days required to reach microbial counts

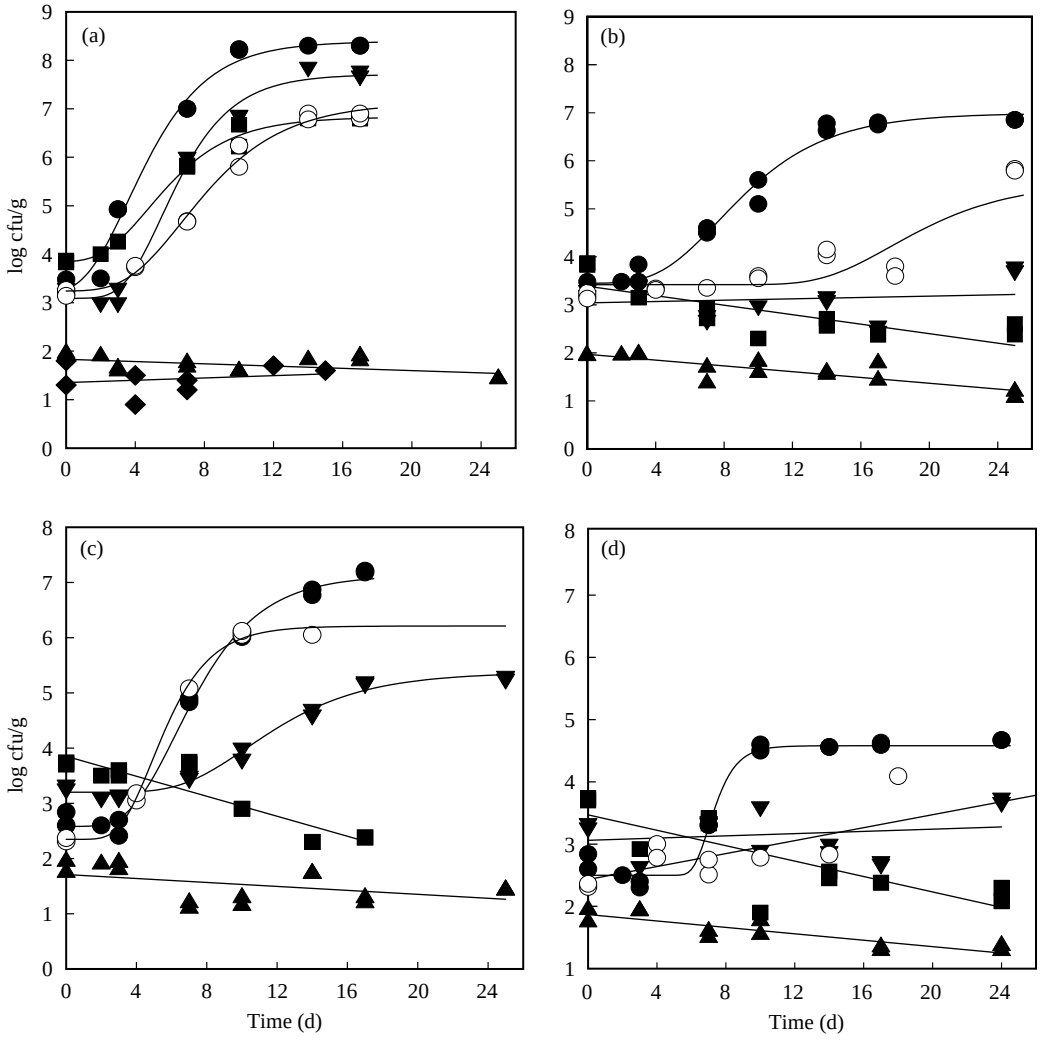

Fig. 5 Mould and yeast counts as a function of storage time at 10 °C. The solid lines correspond to the fitted mathematical models (Gompertz and Linear) for the different tested conditions. (**a,b**) Red Delicious; (**c,d**) Granny Smith. (**a,c**) polyethylene. (**b,d**) ESE film. (**a**,**b**) ●, N1; ▼, N2; ■, N3; ◆, N4; ▲, N5; ○, P3. (**c**,**d**) ●, N10; ▼, N11; ■, N12; ▲, N14; ○, P1

Table 4 Sensory analysis of Red Delicious and Granny Smith apples in formulated unpasteurised orange juice

Apple variety	Juice[a]	Panel score[b]		
		Colour	Texture	Flavour
Red Delicious	N1	3.67 (0.17)	2.78 (0.40)	2.78 (0.32)
	N2	3.00 (0.37)	2.67 (0.37)	2.22 (0.40)
	N3	3.33 (0.24)	2.44 (0.24)	2.44 (0.29)
	N4	3.55 (0.18)	3.22 (0.28)	2.56 (0.24)
	N5	3.33 (0.18)	2.89 (0.33)	2.33 (0.25)
Granny Smith	N10	3.20 (0.25)	3.20 (0.33)	2.90 (0.31)
	N11	3.50 (0.17)	3.30 (0.21)	3.30 (0.15)
	N12	3.40 (0.16)	3.00 (0.33)	2.80 (0.29)
	N13	3.20 (0.20)	3.10 (0.23)	2.70 (0.26)
	N14	3.10 (0.23)	3.30 (0.26)	2.80 (0.20)

[a]Keys to the treatments are in Table 1. A 5-point hedonic scale was used: 0 = dislike extremely; 2 = neither like or dislike; 4 = like extremely. The product was considered unacceptable if it scored below 2.
[b]Standard error within the parentheses. Mean values were calculated with 15 testers.

Table 5 Storage life (d) at 10 °C of apple cubes maintained in formulated orange juices and packaged in plastic films of different gaseous permeability

Apple varieties	Treatment[a]	Storage life (d)	
		Packaging film	
		Polyethylene	ESE
Red Delicious	P3	10.5 (1.5)	> 25
	N1	5 (1.0)	12.5 (2.0)
	N2	7.5 (1.0)	> 15
	N3	8.5 (2.0)	> 15
	N4	10 (1.0)[b]	—
	N5	8.5 (1.0)[b]	> 15
Granny Smith	P1	10.5 (3.0)	> 25
	N10	10.0 (1.5)	> 15
	N11	> 10	> 15
	N12	> 10	> 15
	N14	7 (1.0)[b]	9 (1.0)[b]

Confidence limits within the parentheses.
[a]Keys to the treatments are in Table 1.
[b]Storage life was limited by adverse colour modification.

of $10^{6\pm0.5}$ cfu/g (Howard & Dewi, 1995), a Chromaticity difference $= 6 \pm 0.5$, and Relative softness $\leq -0.6 \pm 0.1$. Values of colour and softness were confirmed by the sensory panel. Selection of the formulations was based on microbial growth parameters, and also on the quality attributes (taste, colour and texture). **Table 5** shows shelf-life values of the tested samples stored at 10 °C; in all cases, these values were higher in the low gaseous permeability film (ESE) than in PE for both apple varieties. RD and GS showed similar shelf-life values when pasteurised juice (P1, P3) and PE packaging were used; under these conditions, deterioration was mainly due to microbial growth. With pasteurised juice and ESE packaging, a shelf-life higher than 25 d was obtained for both varieties, without the need of sorbate or benzoate. The use of unpasteurised orange juice required the addition of potassium sorbate to control microbial growth in RD apples (N2–N5). However, quality was maintained in GS apples during 10 d storage in PE and more than 15 d in ESE without potassium sorbate and only containing sucrose (N10). For RD, shelf-life values of 7.5–8.5 d in PE and more than 15 d in ESE film were obtained with 0.125–0.520 g/kg potassium sorbate (N2–N3). In the case of GS with the same levels of potassium sorbate, shelf-life values were higher than 10 and 15 d in PE and ESE, respectively (N11–N12). All the samples regardless of the packaging film showed microbial levels of total coliforms and sulphite-reducing Clostridium below 2 MPN/mL, indicating safe sanitary conditions in the product.

Conclusions

For Red Delicious (RD) apples, the addition of acidulants (citric acid, or a combination of citric and ascorbic acids) to either pasteurised or unpasteurised orange juice was necessary to obtain an acceptable surface colour in both packaging films (polyethylene, PE and EVA/SARAN/EVA, ESE). Thus, obtained results allowed to replace sodium bisulphite by these acidulants. For Granny Smith (GS) variety in both films, colour was maintained using pasteurised or unpasteurised orange juice without acidulants. Potassium sorbate applied in levels ranging between 0.125 and 0.25 g/kg gave good colour retention; however, higher

concentrations of potassium sorbate (0.375–0.50 g/kg) adversely affected the colour of the apples, even though they had a significant antimicrobial action. Sodium benzoate was discarded as a preservative because it produced severe browning in both apple varieties. Shelf-life values of 10 d in PE and higher than 25 d in ESE film were obtained for both apple varieties in pasteurised juices at 10 °C. For RD in fresh orange juice with acidulants and potassium sorbate (0.125–0.250 g/kg), shelf-life was 7.5–8.5 d in PE and higher than 15 d in ESE film. Shelf-life values of 10 d in PE and higher than 15 d in ESE were obtained for GS in fresh juice without potassium sorbate. All the samples (packaged in both films) showed microbial levels of total coliforms and sulphite-reducing Clostridium below 2 MPN/mL, which corresponded to a product with good sanitary conditions.

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