

# Effect of oxygen and fluorescent light on the quality of orange juice during storage at 8°C

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The effect of oxygen and light exposure and the combination of these two parameters on the retention of ascorbic acid, occurrence of non-enzymatic browning and the formation of 5-hydroxymethyl-2-furfuraldehyde (HMF) and furfural was investigated in orange juice stored at 8°C for 52 days. Orange juice, reconstituted and HTST-pasteurised, was stored in glass containers, covered on the sides with aluminium foil and sealed on the top with packaging materials, i.e. glass, polyethylene and paper carton, having different light transmission and oxygen permeation characteristics. The ascorbic acid content was significantly affected by the level of dissolved oxygen in the juice, but no effect of light could be observed under the conditions of this study. Browning increased during the first 3 weeks of storage in all samples and was over the whole period of storage significantly correlated to the level of dissolved oxygen, but the effect of light was insignificant. HMF and furfural content did not increase significantly in any of the samples during the 52-day storage at 8°C.

## INTRODUCTION

Orange juice holds the leading position among juices in global consumption. The nutrient quality of orange juice is related mainly to its ascorbic acid content, and the decomposition of ascorbic acid and non-enzymatic browning are reported as the major deteriorative reactions occurring during storage. 5-Hydroxymethyl-2-furfuraldehyde (HMF) is produced in orange juice from sugars by heating during processing and can cause browning reactions with amino compounds and sugars. Ascorbic acid is decomposed to furfural, which is known to undergo polymerisation and, as an active aldehyde, may combine with amino acids and contribute to the browning of the juice. Both furfural (Nagy & Randall, 1973; Nagy & Dinsmore, 1974; Kaanane *et al.*, 1988) and HMF (Robertson & Samaniego, 1986; Lee & Nagy, 1988) are useful indicators of storage temperature abuse in commercially processed citrus juices.

Temperature, the presence of oxygen, exposure to

light and the length of storage are the most critical factors influencing the quality of citrus juices. Increasing storage temperature of citrus juices results in increased degradation of ascorbic acid (Nagy, 1980; Graumlich *et al.*, 1986; Kaanane *et al.*, 1988; Kennedy *et al.*, 1992), and non-enzymatic browning in orange concentrate has also been shown to be temperature-dependent (Kanner *et al.*, 1982).

The adverse effect of dissolved oxygen on the quality of citrus juices has been reported by many researchers: increased degradation of ascorbic acid (Bissett & Berry, 1975; Trammell *et al.*, 1986; Kacem *et al.*, 1987*a,b*; Sizer *et al.*, 1988; Kennedy *et al.*, 1992) and increased browning (Robertson & Samaniego, 1986; Trammell *et al.*, 1986; Kacem *et al.*, 1987*b*). Based on sensory evaluation, however, the level of dissolved oxygen did not affect the sensory quality of orange juice stored at 25°C (Trammell *et al.*, 1986; Kacem *et al.*, 1987*a*).

The effect of light has been less investigated and findings are contradictory. Ahmed *et al.* (1976) attributed the flavour changes and ascorbic acid losses in non-pasteurised refrigerated orange juice to light exposure in combination with microbial growth and the presence

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of oxygen in the juice. Fluorescent light had a significant effect on the ascorbic acid degradation and the sensory acceptability of orange drinks stored at ambient temperature (25–30°C) for 32 days (Sattar *et al.*, 1989). No influence of exposure to sunlight was shown, however, on the ascorbic acid content, non-enzymatic browning and organoleptic properties in orange juice stored for 52 days at either 5 or 20°C (Mottar, 1989).

Traditional materials protect orange juice from oxygen (glass and aluminium foil) and light exposure (aluminium foil). Owing to environmental concerns, new polymeric barriers are now being developed that may expose the product to both oxygen and light during chill-storing. However, to what extent such storage conditions might influence the quality of orange juice has been little investigated. The purpose of this study was to investigate the effect of oxygen and light exposure and the combination of these two parameters on the retention of ascorbic acid, occurrence of browning and the formation of HMF and furfural in reconstituted HTST-pasteurised orange juice stored at 8°C.

## MATERIALS AND METHODS

### Materials and storage conditions

Orange juice reconstituted from frozen concentrate with water to 11.8°Brix was HTST-pasteurised at 95°C for 15 s without deaeration and aseptically packed in 1000-ml Tetra Brik Aseptic cartons. The juice was then transferred under hygienic conditions into autoclaved glass containers of 300 ml. The sides of the containers were covered with aluminium foil and the top was sealed with barriers having different light transmission characteristics and different air permeability. The materials used as barriers were an unbleached carton barrier and two polyethylene (PE) barriers, one opaque showing low light transparency coded as PE(LT) and the other with high light transparency coded as PE(HT). The thickness of the polyethylene materials was 50 µm. Glass was used as a fourth barrier, by placing the hermetically sealed glass containers upside down over a bench, with the glass surface towards the light source. These glass containers were filled to the top, thus allowing no air headspace, while the glass containers with polyethylene and the carton lids were filled so that an air layer (~70 ml) was trapped in the container. The light transmission (T%) of the materials used as light barriers was determined at different wave lengths (λ) with a Hitachi U 3200 spectrophotometer (Table 1). The exposure of all samples to light was made uniform by adjusting the distance of the samples from the light source, which consisted of three 40-W cool, white fluorescent lamps. The average light intensity was 2000 lux as measured by a Lutron LX-101 digital lux-meter. The storage temperature was 8°C and the storage period was 52 days. Samples were also kept in darkness under the same conditions in glass containers with airtight tin lids or carton lids. A single measurement of

Table 1. Light transmission (T%) of the materials used as light barriers at different wavelengths

λ (nm)	Carton	PE(LT)	PE(HT)	Glass
300	0	0	3.4	0.25
400	0	3.6	72.8	83.8
500	0	24.8	82.1	85.7

\*PE(LT) and PE(HT), polyethylene barriers showing low and high light transparencies, respectively.

each quality parameter was performed in juice samples from two replicate containers taken at days 0, 3, 8, 14, 22, 30, 38 and 52.

### Methods of analysis

#### Ascorbic and dehydroascorbic acid

Ascorbic acid content was determined by an HPLC analytical procedure using a Milton Roy VS pump (Milton Roy) at a flow rate of 2.5 ml/min, a Valco injection valve with a 10-µl loop, a 250 × 4.6 mm LiChrNH<sub>2</sub> column, a Waters Model 440 detector (Water Associates Inc.) set at 254 nm, and a Sekonic SS-250F recorder (Sekonic Co.). The mobile phase was 75:25 (v/v) acetonitrile/0.05 M KH<sub>2</sub>PO<sub>4</sub>, pH 5.95 (Tuan & Wyatt, 1987). Orange juice (1 ml) was transferred to a 10-ml volumetric flask and made to volume with distilled water. The diluted orange juice was then filtered through a 0.45-µm filter and kept in ice for a few minutes before being injected into the HPLC. The dehydroascorbic acid content was indirectly quantified after reduction to ascorbic acid by adding L-cysteine-hydrochloride monohydrate (1% w/v of the sample) in the filtered sample. The pH of the sample was adjusted to between 7.0 and 7.5 by addition of NH<sub>3</sub> (25%) and maintained at that pH level for 2 min. The pH was then lowered to the initial value (~1.6) with concentrated HCl, after which the sample was analysed for total ascorbic acid content.

#### Browning

Browning was measured as light absorbance at 420 nm (Meydav *et al.*, 1977) with a Beckman DU-8 spectrophotometer (Beckman Instruments Inc.).

#### Furfural and 5-hydroxymethylfurfural

Furfural and 5-HMF were determined simultaneously with the HPLC method described by Lee *et al.* (1986) with some modifications concerning the elution of furfurals (with methanol, 15% v/v in water) and the mobile phase (methanol:water, 15:85 v/v). For the HPLC analysis, a Beckman HOB pump at a flow rate of 1 ml/min, a Beckman 210A injection valve (Beckman Instruments Inc.), and a Supelco, supelcosil™ LC-18, 250 × 4.6 mm and 5 µm particle size, column (Supelco Inc.) were used. The injection volume of the sample was 20 µl. Detection was performed with a Hewlett Packard 1050 detector set at 280 nm, and a Kipp & Zonen BD 40 recorder was used.

*Dissolved oxygen*

The oxygen dissolved in the juice was measured by a Micro-electrode OM-4 Oxygen-Meter (Microelectrodes Inc.) using a membrane-covered oxygen probe, 3 mm  $\phi$  (Clark type MI-730 oxygen Micro-electrode). The lid was removed from the glass container and the oxygen probe was immediately inserted in the juice. The probe was then slowly stirred to ensure a constant oxygen supply to the sensor. The reading was taken when a steady value was registered (after approximately 3 min).

*Statistical analysis*

Multiple regression analysis was performed by the SYSTAT 5.0 program for WINDOWS to evaluate the effect of dissolved oxygen, storage time and light exposure on ascorbic acid degradation and browning. A three-way analysis of variance (ANOVA) was performed to determine significant differences ( $P < 0.05$ ) among the samples stored in different containers.

**RESULTS AND DISCUSSION**

**Oxygen content**

The amount of oxygen dissolved in the juice before the transfer into the different containers was 1.0 mg/l. In the hermetically sealed glass containers, the amount of oxygen progressively decreased until the 30th day, when no oxygen was detected in the juice (Table 2). In the containers with the polyethylene lids, the oxygen reached a maximum value after 3 and 8 days (3.9 and 4.5 mg/l, respectively) and then slowly decreased to a level of about 1.7 mg/l after 22 days of storage. Thereafter, no further changes occurred up to day 52. The carton lid proved to be a poor oxygen barrier (Mar-

**Table 2. Oxygen content in orange juice stored at 8°C in glass containers with different lids<sup>a</sup>**

Storage day	Dissolved oxygen (mg/l)					
	Airtight glass/tin lid		PE lid		Carton lid	
			PE(HT) PE(LT)			
	Light	Dark	Light	Light	Light	Dark
0	1.0	1.0	1.0	1.0	1.0	1.0
3	0.7	1.1	3.9	3.7	7.2	8.8
8	0.7	1.2	2.6	4.5	8.3	9.3
14	2.3	2.4	2.8	3.9	8.7	7.0
22	0.6	0.4	1.8	1.7	5.6	6.7
30	0.0	0.0	1.1	1.8	7.8	7.0
38	0.0	0.0	1.9	1.7	8.2	7.6
52	0.0	0.0	1.8	1.5	7.9	8.3

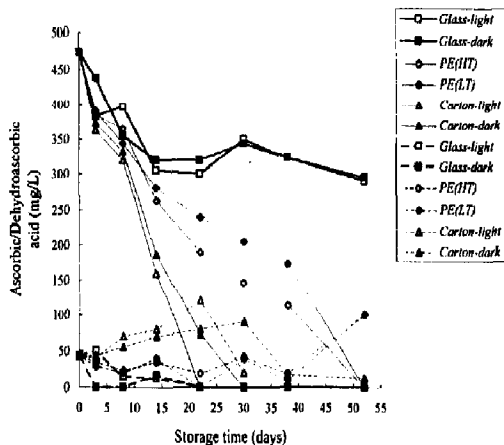
<sup>a</sup>Mean values of single measurements in two replicate samples. Amount of dissolved oxygen in the juice packed in the Tetra Brik Aseptic cartons before transfer to storage containers.

shall *et al.*, 1986), resulting in constantly high levels of dissolved oxygen (from 7 to 9 mg/l) throughout the storage period, corresponding to orange juice saturated with oxygen (Sizer *et al.*, 1988).

**Ascorbic acid**

*Effect of oxygen*

Figure 1 shows the change of ascorbic acid content in samples with different oxygen and light exposure during the 52-day storage period. The ascorbic acid content was 473 mg/l (Table 3) in the juice transferred into the different storage containers at day 0. Within the first week of storage, the ascorbic acid content decreased by about 20% in all samples, which can be explained by the amount of initial oxygen (in the



**Fig. 1. Ascorbic and dehydroascorbic acid changes in orange juice stored at 8°C in glass containers with different lids as affected by storage time. The unbroken lines (—) represent the changes in ascorbic acid and the dashed lines (---) represent the changes in dehydroascorbic acid.**

**Table 3.** Ascorbic acid content of orange juice stored at 8°C in glass containers with different lids\*

Storage day	Ascorbic acid (mg/l)					
	Airtight glass/tin lid		PE lid		Carton lid	
			PE(HT) PE(LT)			
	Light	Dark	Light	Light	Light	Dark
0	473	473	473	473	473	473
3	383 a	438 b	385 a	390 a	363 a	373 a
8	397 a	355 a	365 a	344 a	320 a	332 a
14	305a,b	320 b	263 c	280 a,c	160 d	187 d
22	300 a	320 a	190 b	245 c	0 d	73 e
30	350 a	344 a	147 b	205 b	0 c	0 c
38	323 a	323 a	113 b	173 c	0 d	0 d
52	290 a	295 a	0 b	0 b	0 b	0 b

\*Mean values of single measurements in two replicate samples. Values within the same row followed by different letters (a-c) are significantly different ( $P < 0.05$ ).

headspace, dissolved in the juice and incorporated into the product during the transfer from the cartons into the glass containers) and is in accordance with results reported in previous storage studies (Nagy & Smoot, 1977; Kennedy *et al.*, 1992). After 14 days of storage, the ascorbic acid content was significantly higher ( $P < 0.05$ ) in the hermetically sealed (airtight) glass containers than in the glass containers with polyethylene and carton lids, and significantly higher in the containers with the polyethylene lids than those with the carton lids (Table 3). No further degradation of ascorbic acid was observed in the containers with airtight lids, while the ascorbic acid content was reduced to 0 after 30 days and 52 days in the containers with carton and polyethylene lids, respectively. Multiple regression analysis of the data indicated that ascorbic acid degradation was significantly correlated ( $P < 0.001$ ,  $r^2 = 0.78$ ) to the level of dissolved oxygen and to the length of storage. Similar findings have been reported by Trammell *et al.* (1986), Kennedy *et al.* (1992) and Kacem *et al.* (1987a).

#### Effect of light

Table 3 shows that there is no significant difference ( $P > 0.05$ ) in ascorbic acid degradation in the juice stored in glass containers with airtight lids, exposed and unexposed to light. In the juice samples covered with carton lids, ascorbic acid content decreased to low levels at the same rate for both light-exposed and unexposed samples. Only in juice samples covered by different polyethylene lids were some differences observed on the 22nd and 38th day of storage. The ascorbic acid retention was significantly higher in the samples covered by the opaque polyethylene lid (PE(LT)) than in the samples covered with the transparent polyethylene lid (PE(HT)). However, after 52 days of storage, no ascorbic acid remained in any of these samples. It appears that light may make a slight contribution to the rate of ascorbic acid degradation when there are medium

levels of dissolved oxygen (~2 ppm) in the juice. However, in spite of the observations on the 22nd and 38th day of storage, regression analysis of the data showed that the overall effect of light was insignificant ( $P > 0.1$ ) when juice was stored at 8°C. It can be concluded that light has a minor effect on the degradation of ascorbic acid, compared with that of oxygen, under the conditions of this study. These results are in agreement with those of a previous study, in which sunlight had a minimal influence on the quality of orange juice stored for three months at 5 or 20°C (Moitar, 1989).

#### Dehydroascorbic acid

Dehydroascorbic acid (DHA) is formed from ascorbic acid under aerobic conditions. The orange juice stored in hermetically sealed glass containers for 14 days contained DHA at a level of 4–8% of the total vitamin C content (15–40 mg/l), which is in agreement with the values presented by Nagy (1980). From the 22nd day until the end of storage, DHA was reduced to non-detectable levels (<5 mg/l), while no oxygen was detected in the juice after the same period of storage. The percentage of vitamin C in the form of DHA was higher in the juice samples stored in the containers with the polyethylene lids and even higher in the case of the containers with the carton lids (Fig. 1). In these samples, the oxidation of ascorbic acid to DHA was extensive, causing a significant and continuous loss of vitamin C. Regression analysis showed a significant effect of dissolved oxygen ( $P < 0.01$ ,  $r^2 = 0.17$ ) on the formation of dehydroascorbic acid.

#### Browning

Browning took place at the same rate in all samples during the first 3 weeks of storage (Fig. 2). After the 30th day of storage, browning seemed to level off in all samples. However, after 52 days of storage, the samples with the carton lids showed a significantly higher ( $P < 0.05$ ) browning value than the other samples. These results correlate with those of Mannheim *et al.* (1987), who found that initially small differences in the rate of browning in orange juice stored in cartons and in glass containers became greater with increased storage time during 12 weeks of storage at ambient temperature (25°C). Regression analysis indicated that browning was significantly ( $P < 0.001$ ,  $r^2 = 0.77$ ) correlated to the level of dissolved oxygen and time of storage, but the effect of light was insignificant ( $P > 0.05$ ). The correlation between browning and the level of dissolved oxygen may be a result of the oxidative degradation of ascorbic acid. Increased levels of dissolved oxygen result in increased oxidation of ascorbic acid, and thus increased development of brown pigments in the juice. The relation between browning and ascorbic acid loss in orange juice during storage has been pointed out by other researchers (Trammell *et al.*, 1986; Kacem *et al.*, 1987a,b). Light has previously been shown to have a minor effect on browning during the

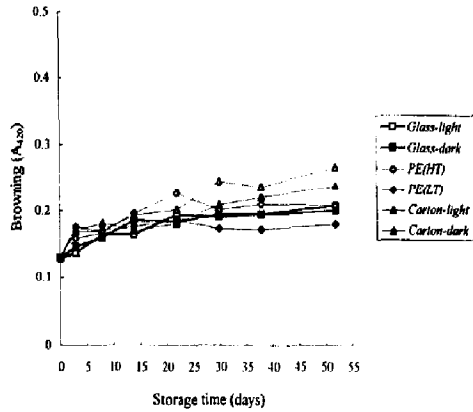


Fig. 2. Non-enzymatic browning of orange juice stored at 8°C in glass containers with different lids as affected by storage time.

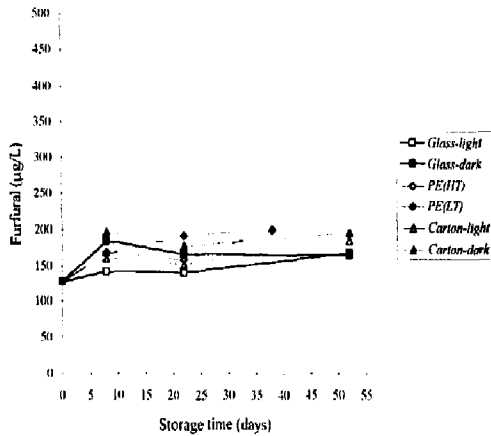


Fig. 3. Furfural accumulation in orange juice stored at 8°C in glass containers with different lids as affected by storage time.

storage of orange juice in different containers and different light conditions (Motar, 1989).

#### HMF and furfural accumulation

The amount of HMF was low in all samples (<100 µg/l) during the 52-day storage period and close to the detection limit of the method. Furfural content increased slightly (from 128 to 200 µg/l), but no significant differences could be observed between the different samples (Fig. 3). These results are in agreement with previous data that indicate a slow increase of HMF levels in grapefruit juice stored at 10°C (Lee & Nagy, 1988) and of furfural levels in orange juice stored at 5 and 10°C (Nagy & Randall, 1973). Kanner *et al.* (1982)

have earlier shown that 12°C seems to be a critical storage temperature, below which furfural accumulation is very slow.

#### CONCLUSIONS

The level of dissolved oxygen and the length of storage were the most important factors for the degradation of ascorbic acid and browning in orange juice stored at 8°C. No effect of light on the degradation of ascorbic acid and browning could be observed under the conditions of this study. HMF and furfural content did not increase during storage at 8°C and no significant differences were observed between the different samples.

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