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Changes in vitamin C and flavour components of mandarin juice due to curing of fruits

Ana G. Pérez *, Pilar Luaces, Jesus Oliva, Jose J. Ríos, Carlos Sanz

Department of Physiology and Technology of Plant Products, Instituto de la Grasa, CSIC, Padre García Tejero, 4, 41012-Seville, Spain

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Abstract

Changes in vitamin C and flavour components of mandarin juice induced by previous curing (intermittent heat treatment at 38 °C and 97% relative humidity) of fruits were studied. Acetaldehyde and ethanol accumulation were enhanced by intermittent curing (IC) of fruits, though the final contents of both compounds in juice were below levels associated with off-flavour development. IC seems to have opposite effects in relation to limonene and linalool oxidation in fruits stored at 20 and 5 °C. Thus, IC produces a 20% reduction in limonene content at room temperature while it induces a 20–30% accumulation in cold-stored fruits. Quantification of the main terpene-oxidised compounds: α-terpineol, carvone and *E*-carveol did not indicate any detrimental effect of IC on fruit aroma under the studied conditions. Non-significant differences were found either for main sugars or organic acids. Negative effects were found in relation to vitamin C retention, with a 30% greater loss in the juice of IC-treated fruits.

Keywords: Flavour; Aroma; Vitamin C; Curing; Mandarin; Juice

1. Introduction

Synthetic fungicides have been the main method to control green (*Penicillium digitatum* Sacc) and blue mould (*Penicillium italicum*) development during citrus post-harvest life. Nevertheless, there is growing international concern over the indiscriminate use of these chemicals on food crops because of the possible harmful effects on human health. Thus, there is a critical need for the development of reliable and environmently friendly methods for protecting perishable crops, and particularly fresh fruits, against losses after harvest. Achieving high levels of control with a single system is difficult and the use of an integrated rather than a single approach is advocated. In this sense, the potential of high temperature *post-harvest* treatments has been successfully assayed in combination with additional technologies, such as cold-storage, controlled atmospheres or biological control, for different citrus fruits (Couey, 1989; Obagwu & Korsten, 2003). Heat-treatments induce accumulation of antifungal compounds and cause inhibition of pathogen infection (Ben-Yehoshua, Barak, & Shapiro, 1987; Porat et al., 2000). Holding citrus fruits at 32–38 °C and 94–98% RH for 2–3 days (curing) has been described as an effective way to prevent decay on oranges stored at different temperatures (Plaza et al., 2003).

Though quality of mandarin is generally more sensitive to storage than other citrus varieties, possibly due to their relatively short maturation period, synergistic effects against chilling injury and decay have also been obtained in some mandarin cultivars by combining heat treatments (vapour heat, curing and hot-water dipping) with other storage techniques (Rodov, Ben-Yehoshua,

* Corresponding author. Tel.: +34-95-4611550; Fax: +34-95-4616790.
E-mail address: agracia@cica.es (A.G. Pérez).

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Albagli, & Fang, 1995; Schirra & Mulas, 1995). Our group have been working on new mild physical post-harvest treatments for orange and mandarin fruits, compatible with biological control systems. Among the physical treatments assayed, several temperatures have been tested in traditional and intermittent curing (IC) procedures.

Mandarin flavour, involving fewer volatile components than oranges, is very easily affected by heat treatments and relatively larger increases in off-flavour compounds than in oranges have been reported (Hagenmeier & Shaw, 2002; Shaw, Moshonas, Nisperos-Carriedo, & Carter, 1992). In fact, mandarin juice products have been difficult to market because of certain off-flavours as well as changes in flavour that occur during storage (Moshonas & Shaw, 1997). The habitual practice of adding mandarin juice to reinforce colour of commercial orange juices makes even more important those studies directed to improve mandarin flavour quality. Thus, despite the effectiveness in pathogen control, additional data on volatile composition, sugar-acid contents, and vitamin C levels are needed to fully evaluate the benefits and/or detrimental effects of any new post-harvest treatment applied to mandarin fruits.

Therefore, the purpose of the present study, was to document modifications of vitamin C and flavour components of mandarin juice induced by IC of fruits and subsequent storage at different temperatures.

2. Materials and methods

2.1. Fruit samples

The Mandarin variety used in this study was Hernandina, an offshoot of a Clementine variety (*Citrus reticulata blanco* cv. Fina) released in 1983 (Bono-ubeda, de Córdoba-O'Connor, & Soler-Aznar, 1983) that accounts for 8% of Spanish mandarin production. Fruits were grown in Rio Tinto (Huelva, Spain) following standard culture practices, harvested in December and, without any commercial post-harvest treatments, transported to the laboratory. Four batches, 160 fruits each, were randomly established in order to test four different storage conditions: cold storage at 5 °C, storage at room temperature (20 °C), intermittent curing plus cold storage (IC-cold) and intermittent curing plus storage at room temperature (IC-roomT). IC was carried out in a cell at 97 ± 1% RH in which a three temperature cycle was programmed: 18 h at 38 °C followed by 6 h at 20 °C and finally 18 h at 38 °C.

To evaluate the efficacy of the treatments to control fruit decay, 80 naturally infected fruits per batch were selected, and incidence of disease evaluated at the end of storage.

For nutritional and flavour evaluation studies, each sampling day, 20 fruits per treatment were sliced into halves and juiced with an electric juice maker with rotating head. Four juice samples were taken per treatment, each pooled from five pieces of fruit.

2.2. Analysis of volatile compounds

Four vials containing 3 ml of juice, and 3 ml of an aqueous saturated $CaCl_2$ solution immediately added after juice extraction, were analysed for each treatment and day. Juice samples were placed in a vial heater at 40 °C. After 10 min equilibrium time, volatile compounds were adsorbed on a SPME fibre DVB/Carboxen/PDMS 50/30 μm. Sampling time was 25 min at 40 °C. Desorption of volatile compounds trapped in the SPME fibre was done directly into the GC injector. Volatiles were analysed using a HP-5890 gas chromatograph equipped with fused silica capillary column DB-Wax (30 m×0.25 mm). Quantification, expressed in ng/ml of juice (ppb) was performed using individual calibration curves for each identified compound. Those curves were obtained by adding known amounts of the corresponding volatile compounds to a water solution with a sugars/acid composition similar to orange juice, those being standard solutions analysed as previously described. Compound identification was carried out on a HRGC-MS Fison series 8000 equipped with a similar stationary phase column and two different lengths, 30 and 60 m, matching against the Wiley/NBS Library and by GC retention time against standards. On each sampling day, four juices per treatment were analysed.

2.3. Sugar and organic acids analysis

Juice was vacuum-filtered through Whatman No. 1 filter paper and 30 ml of the filtrate were loaded onto a Sep-Pack C18 cartridge (Lida, Kenosha, WI) for pigment clean up. These extracts, containing sugars and organic acids, were filtered through 0.45 μm Nylon filters before HPLC analysis. Sugars and organic acids were analysed in a Beckman liquid chromatograph equipped with a photodiode array detector and a Waters 410 differential refractometer connected in series. Isocratic separations of the compounds were done on a stainless steel Ion-300 (300×7.8 mm, 10 mm) column according to Pérez, Olías, Espada, Olías, and Sanz (1997). On each sampling day, four extracts per treatment were analysed.

3. Results and discussion

Thirty-two volatile compounds were identified and quantified in juice obtained from fresh harvested Hernandina fruits (day 0 in Tables 1 and 2). Ethanol was by far the most abundant compound in fresh fruits, fol-

Table 1
Volatile compounds found in Hernandina mandarins subjected to IC at 38 °C, and subsequent cold-storage

	Day 0	Day 4, cold-storage	Day4, IC-cold	Day 9, cold-storage	Day 9, IC-cold
Acetaldehyde	4820 ± 471	9025 ± 3324	22789 ± 449	12107 ± 515	26219 ± 2157
Ethyl acetate	22 ± 4	34 ± 12	88 ± 18	31 ± 2	73 ± 3
Ethanol	94110 ± 22554	137799 ± 20814	770358 ± 87069	288430 ± 4840	793882 ± 48249
Methyl butanoate	4 ± 2	3 ± 0	3 ± 0	6 ± 1	5 ± 1
α-Pinene	273 ± 60	400 ± 15	566 ± 146	256 ± 50	32372 ± 3998
Ethyl butanoate	7 ± 1	13 ± 2	11 ± 3	13 ± 1	15 ± 1
Ethyl 2-methylbutanoate	0	3 ± 0	5 ± 0	0	4 ± 0
β-Pinene	133 ± 18	139 ± 25	145 ± 11	324 ± 75	332 ± 72
Sabinene	158 ± 53	198 ± 59	393 ± 19	144 ± 54	198 ± 8
3-Carene	23 ± 2	58 ± 21	99 ± 15	66 ± 6	81 ± 12
Myrcene	470 ± 90	831 ± 70	1048 ± 243	468 ± 167	492 ± 111
d-Limonene	34516 ± 2657	37754 ± 3529	45228 ± 10124	19504 ± 343	26192 ± 4901
Ethyl hexanoate	2 ± 0	1 ± 0	12 ± 3	4 ± 1	14 ± 4
Octanal	9 ± 2	38 ± 12	64 ± 9	25 ± 3	44 ± 6
Hexanol	11 ± 2	15 ± 5	11 ± 1	21 ± 3	17 ± 1
Nonanal	6 ± 1	4 ± 1	4 ± 1	3 ± 0	5 ± 1
1,8-Octanodiol	2 ± 0	1 ± 0	1 ± 0	2 ± 0	2 ± 0
Ethyl octanoate	2 ± 1	3 ± 1	2 ± 1	4 ± 0	1 ± 0
Formyl octanoate	2 ± 1	5 ± 1	1 ± 0	1 ± 0	2 ± 0
Decanal	14 ± 1	24 ± 10	37 ± 3	22 ± 3	43 ± 2
Linalool	1058 ± 172	5746 ± 731	4353 ± 1111	2391 ± 457	2152 ± 274
Octanol	12 ± 1	58 ± 16	37 ± 3	24 ± 9	13 ± 3
1-Terpen- 4- ol	107 ± 6	302 ± 21	215 ± 83	118 ± 45	141 ± 17
p-Mentha-*cis*-2,8-diene-1-ol	19 ± 1	266 ± 45	220 ± 32	266 ± 19	220 ± 23
p-Mentha-*trans*-2,8-diene-1-ol	23 ± 3	119 ± 32	101 ± 21	138 ± 29	55 ± 20
α-Terpineol	197 ± 27	853 ± 34	908 ± 24	605 ± 46	587 ± 79
Carvone	6 ± 1	11 ± 1	19 ± 4	11 ± 1	20 ± 1
cis-Carveol	30 ± 2	193 ± 23	64 ± 22	110 ± 23	55 ± 7
Citronellol	50 ± 12	266 ± 12	174 ± 23	183 ± 22	119 ± 12
trans-Carveol	1 ± 0	37 ± 11	73 ± 21	101 ± 17	92 ± 9
Isopiperitenol	51 ± 1	101 ± 21	165 ± 12	128 ± 24	193 ± 13
Nerol	20 ± 2	36 ± 8	30 ± 9	29 ± 3	29 ± 6

Amounts expressed as ng/ml of juice (ppb).
* Values are means and standard deviations of four analyses.

lowed by limonene. Appreciable amounts of other terpene compounds, such as β-pinene, sabinene, myrcene, linalool or α-terpineol, were also detected while lower than expected levels of ethyl esters were found. According to published data on orange juice (Ahmed, Dennison, & Shaw, 1978; Hinterholze & Schieberle, 1998) acetaldehyde, hexanal, octanal, dodecanal and limonene are the most important contributors to orange flavour, with ethyl esters and linalool as additional compounds. Most mandarin cultivars studied so far (Moshonas & Shaw, 1997; Shaw, Moshonas, & Nisperos-Carriedo, 1992) seem to have low levels of those volatile constituents identified as important for citrus flavour when compared to orange values. In this sense, the Hernandina juice profile seems to be comparable to those of mandarin varieties Dancy and Murcott, described by Moshonas and Shaw (1997) as having relatively high levels of volatile compounds. Low-volatility oil-soluble constituents believed to contribute to mandarin flavour (methyl-*N*-methylanthranilate, thymol, and α-sinensal) were not volatile enough to be quantified by this head-space method.

The effectiveness of curing in preventing blue mould development in oranges has recently been described (Plaza et al., 2003) and similar results have been obtained in our laboratory with different mandarin cultivars. In this study, in which our main objective was to evaluate nutritional and flavour modifications, IC was also demonstrated to be a useful treatment for preventing fruit decay with significantly lower percentages of infected fruits in IC-cold (2%) and IC-roomT (4%) than in cold-stored (5%) or room-stored fruits (25%). To determine whether this alternative treatment for decay control could affect the quality of mandarin juice, studies on volatile compounds, sugars, organic acids and vitamin C contents were carried out.

In order to evaluate aroma changes induced by IC under different storage conditions, cold-storage (Table 1) and room temperature (Table 2), both the key-aroma compounds and the off-flavour related compounds present in mandarin juice were studied. Acetaldehyde, ethanol and ethyl acetate are well known indicators of off-flavour in fruits (Sanz, Pérez, Olías, & Olías, 1999), though acetaldehyde and ethyl acetate have also been

Table 2
Volatile compounds found in Hernandina mandarins subjected to IC at 38 °C, and subsequent storage at room temperature

	Day 0	Day 4 20 °C	Day4 IC-roomT	Day 9 20 °C	Day 9 IC-roomT
Acetaldehyde	4820±471	13178±604	23643±807	13606±920	27946±1301
Ethyl acetate	22±4	47±9	138±80	49±11	75±7
Ethanol	94110±22554	331939±2544	978159±71	247190±58090	676186±44175
Methyl butanoate	4±2	3±0	3±0	6±1	6±1
α-Pinene	273±60	601±181	604±76	32863±	278±56
Ethyl butanoate	7±1	14±2	11±2	15±2	15±4
Ethyl 2-methylbutanoate	0	3±0	5±2	3±0	4±1
β- Pinene	133±18	114±29	97±26	336±8	318±40
Sabinene	158±53	439±63	368±81	134±21	182±9
3- Carene	23±2	90±7	94±24	63±32	64±7
Myrcene	470±90	1185±330	880±198	578±161	442±80
d-Limonene	34516±2657	50714±14261	41884±8241	28416±7264	22960±3861
Ethyl hexanoate	2±0	3±0	12±7	2±1	11±3
Octanal	9±2	77±5	63±1	29±7	21±4
Hexanol	11±2	9±1	9±1	18±2	19±1
Nonanal	6±1	3±0	7±20	3±0	4±1
1,8-Octanodiol	2±0	1±0	1±0	2±0	2±1
Ethyl octanoate	2±1	3±2	1±0	1±0	5±1
Formyl octanoate	2±1	4±0	1±0	1±0	2±1
Decanal	14±1	61±0	22±3	27±2	29±5
Linalool	1058±172	5881±354	1544±177	3239±153	1985±287
Octanol	12±1	61±0	16±8	27±15	13±3
1- Terpen- 4- ol	107±6	453±13	262±39	142±6	88±10
p-Mentha-*cis*-2,8-diene-1-ol	19±1	238±31	220±15	248±16	229±19
p-Mentha-*trans*-2,8-diene-1-ol	23±3	138±21	138±18	101±32	64±3
α-Terpineol	197±27	1146±98	807±34	917±81	532±34
Carvone	6±1	13±2	23±6	13±3	15±1
cis -Carveol	30±2	220±38	128±11	174±38	73±4
Citronellol	50±12	312±31	211±27	211±26	119±13
trans-Carveol	1±0	83±21	156±19	83±15	83±9
Isopiperitenol	51±1	101±13	312±28	110±23	165±23
Nerol	20±2	36±4	25±5	36±5	100±23

Amounts expressed as ng/ml of juice (ppb).
* Values are means and standard deviations of four analyses.

reported as desirable compounds for citrus aroma. Under all storage conditions assayed, the acetaldehyde levels of Hernandina fruits increased steadily from fresh-harvested fruits (4,820 ppb) to the end of the experiment. Cold-stored fruits showed a very similar value (12,107 ppb) to room-stored fruits (13,606 ppb) while Hernandina fruits subjected to intermittent curing (IC-cold and IC-roomT) showed a substantial increase, duplicating that amount. Ethanol content showed a quite similar profile to that described for acetaldehyde, with a 2 or 3-fold higher content in juices obtained from IC-treated fruits than in control fruits (Tables 1 and 2). Similar results on the effect of heat treatments on ethanol build-up in tangerines have been reported (Cohen, Shalom, & Rosenberger, 1990). By comparing data listed in Tables 1 and 2, it is clear that accumulation of both compounds, acetaldehyde and ethanol, is more affected by IC treatment than by storage temperature. Amounts of ethyl acetate detected in this study were very low after any treatment and days of storage. None of the three mentioned compounds in juices of IC-roomT or IC-cold fruits reached a final content that could be considered indicative of off-flavour development according to data reported in previous studies (Baldwin, Nisperos-Carriedo, Shaw, & Burns, 1995; Cohen et al., 1990; Hagenmeier & Shaw, 2002). One of the most important reactions involved in citrus aroma alteration during storage is terpene oxidation (Haleva-Toledo, Naim, Zehavi, & Rouseff, 1999). It is well known, for example, that limonene oxidation gives rise to 17 compounds. In this sense it is important to take into account that some key aroma compounds, such as limonene, linalool or valencene, can also be defined as off-flavour precursors, giving rise to detrimental compounds, such as α-terpineol, carvone or *E*-carveol (Duerr & Schobinger, 1981). On day 9, in all stored fruits, limonene and linalool contents were lower than at day 0 (Tables 1 and 2). The observed decreasing levels, more notable in the case of limonene, followed a common pattern with an initial slight increase on day 4. In relation to these two compounds, CI seems to have opposite effects on room temperature-and cold-stored fruits. CI produces a 20% reduction in limonene content at room temperature (Table 2) while it induces a 20–30% accumulation in

cold-stored Hernandina fruits (Table 1). Despite the marked decrease in linalool content of IC-roomT stored fruits on day 4, significant differences among treatments were found in linalool contents at the end of the storage (Tables 1 and 2). In a recent study, no differences in linalool concentrations due to heat treatment were found in hand-made orange juices (Bazemore, Rouseff, & Naim, 2003). The conversion of limonene and linalool to α-terpineol is quite complex; α-terpineol is simultaneously formed and degraded, especially at temperatures above 35 °C (Haleva-Toledo et al., 1999). Linalool is a more reactive substrate than limonene for producing α-terpineol: however, since there is more limonene than linalool in citrus juices α-terpineol appeared to be formed from both precursors. α-Terpineol was the most abundant detrimental oxidized compound found in all juices, increasing up to 3 or 5 fold from 197 ppb in fresh fruit juice. In this study, the fruits stored at room temperature showed the highest levels of the two precursors, limonene and linalool, and also accumulated a significant 40% higher amount of α-terpineol (917 ppb). This final concentration is far from the calculated odour threshold for this compound (2.5 mg/l) that can easily be reached in orange juice stored for 2 weeks at 45 °C (Haleva-Toledo et al., 1999). Curiously, juice obtained from IC-roomT fruits showed (at the end of the storage) a significantly lower content of oxidized compounds than juice from room-stored fruits (Table 2). A protective effect of heat treatment, by interrupting the continuous oxidation process taking place at ambient temperature, is inferred. Quantification of the main compounds formed by this terpene oxidation process, α-terpineol, carvone and *E*-carveol, does not indicate any detrimental effect of IC under the studied conditions.

Sugar content showed a slight decrease during the storage period in all fruits (Table 3). In relation to sucrose levels, though non-significant differences were found at the end of storage, a significantly higher sucrose content was found on day 4 in juices obtained from IC-treated fruits. No significant differences in glucose or fructose contents were found among treatments. In this sense, no effect of curing on soluble solids levels has been found but an increase in the SSC: TA ratio seems to be associated with citrus heat treatment (Plaza et al., 2003; Shellie, Firko, & Mangan, 1993). In fact, opposite influences of heat treatment on TA have been reported by Shellie et al. (1993), with higher TA at higher temperature yet lower TA over storage time at a high temperature. In our study, a steady decrease in citric acid levels was observed during storage, and no effect on citric and malic acid contents could be attributed to IC (Table 3). The most important differences among treatments were found in relation to retention of vitamin C. Vitamin C (ascorbic acid) is the major antioxidant compound found in citrus fruits (Gardner, White, McPhail, & Duthie, 2000). Vitamin C retention has been used as a quality indicator during shelf-life of citrus-derived products (Lee & Coates, 1999). In our study, from the highest value found in juice of fresh-harvested fruits (0.42 mg/ml), vitamin C levels decreased under all storage conditions (Table 3), with significantly lower values in IC-roomT stored fruits on day 4 (0.37 mg/ml) and day 9 (0.26 mg/ml). This observed 30% greater loss of ascorbic acid at the end of IC-roomT storage could be explained by the thermal instability of ascorbic acid. In this sense, in our previous studies carried out on strawberry fruits, we have observed a clear relationship between vitamin C content and storage conditions, with a good retention of ascorbic acid and even a slight initial increase after 10 days of storage at 2 °C (Sanz, Olías, & Pérez, 2002). More recently, Sánchez-Moreno, Plaza, De ancos, and Cano (2003) reported that, for processes combining high-pressure and heat treatment, the highest ascorbic acid retention occurred in orange juices stored at 4 °C and the content progressively decreased with increase in the storage temperature.

In conclusion, no detrimental effect on mandarin flavour was detected under the studied conditions. No overproduction of off-flavour related compounds or

Table 3
Sugar, organic acid and vitamin C contents of Hernandina mandarins subjected to IC at 38 °C, and subsequent storage at 5 °C and 20 °C

		Sucrose	Glucose	Fructose	Citric acid	Malic acid	Vitamin C
Day 0		57.2 ± 0.27	20.8 ± 2.52	22.0 ± 0.10	8.51 ± 0.21	2.09 ± 0.03	0.42 ± 0.05
Day 4	Cold-storage	56.5 ± 0.97	20.4 ± 0.58	21.5 ± 0.66	7.74 ± 0.21	2.10 ± 0.13	0.41 ± 0.02
	IC-cold	60.5 ± 0.83	21.8 ± 1.29	20.6 ± 2.41	8.65 ± 0.01	2.18 ± 0.19	0.42 ± 0.04
	20 °C	57.1 ± 0.27	20.7 ± 0.38	21.1 ± 0.03	8.59 ± 0.44	2.01 ± 0.06	0.39 ± 0.00
	IC-roomT	60.8 ± 1.19	22.3 ± 0.35	20.7 ± 0.30	9.07 ± 0.59	2.28 ± 0.01	0.37 ± 0.00
Day 9	Cold-storage	55.1 ± 1.59	19.7 ± 0.52	20.4 ± 0.54	7.94 ± 0.66	1.98 ± 0.01	0.35 ± 0.05
	IC-cold	57.6 ± 2.98	20.2 ± 2.51	19.0 ± 2.21	7.57 ± 0.03	1.88 ± 0.23	0.36 ± 0.04
	20 °C	55.7 ± 0.31	21.3 ± 0.42	22.1 ± 0.11	8.01 ± 1.14	2.25 ± 0.02	0.38 ± 0.02
	IC-roomT	54.0 ± 0.04	18.9 ± 0.69	18.2 ± 1.28	7.05 ± 0.12	1.97 ± 0.13	0.26 ± 0.00

Amounts expressed as mg/ml of juice.
* Values are means and standard deviations of four analyses.

excessive accumulation of detrimental compounds formed by terpene oxidation was observed. Similarly, no significant differences in sucrose, glucose, fructose or malic and citric acid contents could be attributed to IC treatment. The main objection to IC is the increased loss of vitamin C observed in fruits that are further stored at ambient temperature. This negative effect was not observed in IC-cold stored fruits.

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