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Effects of different drying treatments on the stability of carotenoids in Taiwanese mango (*Mangifera indica* L.)

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Abstract

The stability of carotenoids in Taiwanese mango as affected by different drying treatments was studied. Mangoes were soaked in 1% sodium hydrogen sulfite solution or 1% ascorbic acid solution, prior to hot-air drying and freeze-drying. Results showed that in most cases, the highest yield of the epoxy-containing carotenoids was achieved by freeze-drying plus soaking in 1% sodium hydrogen sulfite solution. However, freeze-drying plus soaking in 1% ascorbic acid solution resulted in the highest retention of all-*trans*-β-carotene and its *cis* isomers, all-*trans*-zeaxanthin and its *cis* isomers, as well as *cis*-lutein. Nevertheless, for hot-air drying, with or without soaking, a mango product of deep orange colour was produced. On freeze-drying, mango could generate yellow colour, while a lighter color was observed when soaked in antioxidants.

Keywords: Taiwanese mango; Carotenoids; Drying treatment; HPLC

1. Introduction

Mango, a major fruit produced during the summer season in Taiwan, has been reported to contain high amounts of carotenoids (Chen, Tai, & Chen, 2004). The significance of consumption of carotenoids for improvement of human health has been well documented (Rao & Agarwal, 1999; Sesso, Buring, Norkus, & Gaziano, 2004). For instance, β-carotene has been shown to possess high vitamin A activity and antioxidative capacity (Mercadante & Rodriguez-Amaya, 1998; Miller, Sampson, Candeias, Bramley, & Rice-Evans, 1996). Codoy and Rodriguez-Amaya (1989) reported that β-carotene is the dominant carotenoid in mango, comprising of 48–84% of the total carotenoid content.

The composition of carotenoids in mango can be affected by many factors, i.e., cultivar, geographical origin, degree of maturity and processing conditions (Cano & de Ancos, 1994; Pott, Marx, Neidhart, Mühlbauer, & Carle, 2003). Mercadante and Rodriguez-Amaya (1998) found that ripening alterations occurred principally in the major carotenoids, violaxanthin and β-carotene. However, only β-carotene remained as the principal carotenoid in commercially processed mango juice, while violaxanthin was not detected (Mercadante & Rodriguez-Amaya, 1998). In a study dealing with the determination of β-carotene stereoisomers in fresh, dried and sun-dried mango, Pott et al. (2003) concluded that drying resulted in a complete and partial degradation of xanthophylls and all-*trans*-β-carotene, respectively. The authors also reported that conventionally dried mangoes were characterized by elevated amounts of 13-*cis*-β-carotene, whereas sun-dried mango slices contained additional amounts of 9-*cis*-β-carotene (Pott et al., 2003).

In Taiwan, dried mango is a popular fruit commodity. The composition of carotenoids in Taiwanese mango has been determined (Chen et al., 2004); however, the stability of carotenoids in Taiwanese mango as affected by different

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drying methods remains unknown. In a previous study, Tai and Chen (2000) found that only 10% carotenoid loss occurred for Daylily flowers when soaked in 1% sodium sulfite solution prior to hot-air drying. However, a 50% loss of carotenoids was shown for the treatment without soaking. The objectives of this study were to determine the stability of carotenoids in Taiwanese mangoes as affected by various soaking and drying treatments.

2. Materials and methods

2.1. Materials

A total of 40 mangoes with an average weight of 750 g each were obtained from a farm located in southern Taiwan, and transported immediately to the laboratory on the same day. All-*trans*-α-carotene, all-*trans*-β-carotene and all-*trans*-lutein standards were purchased from Sigma Co. (St. Louis, MO, USA). Chemicals, including anhydrous sodium sulfate, magnesium oxide and potassium hydroxide were from Riedel-de Häen Co. (Barcelona, Spain). Zeaxanthin standard was prepared from yellow corn using column chromatography as described by Chen et al. (2004). Likewise, both neoxanthin and violaxanthin standards were prepared from spinach by thin-layer chromatography (Chen et al., 2004). The YMC C-30 column (250 × 4.6 mm I.D., 5 μm) used was from Waters Co. (Milford, MA, USA).

2.2. Instrumentation

The HPLC instrument is composed of a degasser (model DP-4010, Sanwa Tsusho Co., Tokyo, Japan), an injector (model 7161, Rheodyne Co., Rohnert Park, CA, USA), two Jasco PU-980 pumps (Jasco Co., Tokyo, Japan) and a Jasco MD-915 photodiode-array detector (Jasco Co.). The freeze-dryer (model FD-24) was from Ching-Ming Co (Taipei, Taiwan). The rotary evaporator (model M-1) was from Eyela Co. (Tokyo, Japan).

2.3. Drying treatment

Mangoes were washed with running water, peeled, seed removed and the pulps were cut into pieces (3 × 9 cm each) with a knife. All the pieces were divided into six portions of 3150 g each and then subjected to the following treatments: (A) mango pieces were dried under hot-air at 60 °C for 20 h, (B) mango pieces were soaked in 1% sodium hydrogen sulfite solution for 30 min prior to treatment A, (C) mango pieces were soaked in 1% ascorbic acid solution for 30 min prior to treatment A, (D) mango pieces were freeze-dried (−53 °C, 0.06 torr) for 32 h, (E) mango pieces were soaked in 1% sodium hydrogen sulfite solution for 30 min prior to treatment D, and (F) mango pieces were soaked in 1% ascorbic acid solution for 30 min prior to treatment D. All the treatments were carried out in triplicate. The drying time was selected based on the final moisture content of each sample, which is approximately 18%.

2.4. Analysis of carotenoids in Taiwanese mango

Dried mango pulps were ground into a fine powder prior to extraction, and a procedure based on Chen et al. (2004) was followed. Briefly, 1 g mango powder was mixed with 30 ml hexane–ethanol–acetone–toluene (10:6:7:7, v/v/v/v) in a 100-ml volumetric flask. After shaking for 1 h, 2 ml of methanolic KOH (40%) was added to the flask for saponification in the dark for 16 h under nitrogen. Then 30-ml hexane was added for extraction of carotenoids. After shaking for 1 min, 10% sodium sulfate solution (in water) was added and diluted to volume. The hexane layer containing carotenoids was collected, and the lower phase was repeatedly extracted. Then all the hexane extracts (upper layer) were combined and evaporated to dryness under vacuum. All the extraction procedures were conducted under dimmed light and nitrogen gas was flushed into the flask to avoid isomerization or degradation loss of carotenoids. The dried residue was dissolved in 1-ml methanol–methylene chloride–isopropanol (89:1:10, v/v/v) and filtered through a 0.2-μm membrane filter for HPLC analysis. An HPLC gradient mobile phase of methanol-isopropanol (99:1, v/v) (A) and 100% methylene chloride (B) as described by Chen et al. (2004) was used: 100% A and 0% B initially, maintained for 15 min, decreased to 70% A in 45 min, maintained for 15 min and returned to 100% A in 65 min. A C-30 column with flow rate at 1.0 ml/min and detection at 450 nm was used. The injection volume was 20 μl. The various carotenoids, including neoxanthin, violaxanthin, zeaxanthin and β-carotene were identified by comparing retention time and absorption spectra with reference standards as well as co-chromatography with added standards. In addition, the epoxide test was carried out by adding a few drops of methanolic HCl to the sample extract for identification of the epoxy-containing carotenoids (Chen et al., 2004). Both absorption spectra and Q-ratios were used for tentative identification of *cis* isomers of carotenoids (Lin & Chen, 2003; Tai & Chen, 2000). The quantification was performed using an internal standard, α-carotene, as reported by Chen et al. (2004). All the treatments were conducted in triplicate. The data were analyzed based on SAS (2003) by using analysis of variance and Duncan's multiple range test.

3. Results and discussion

3.1. HPLC analysis of carotenoids

Fig. 1 shows the HPLC chromatogram of a carotenoid extract from mango after freeze-drying plus soaking in 1% sodium hydrogen sulfite solution. Most peaks showed adequate resolution. α-Carotene was found to be a suitable internal standard to quantify all the carotenoids because it did not interfere with separation of the other carotenoids.

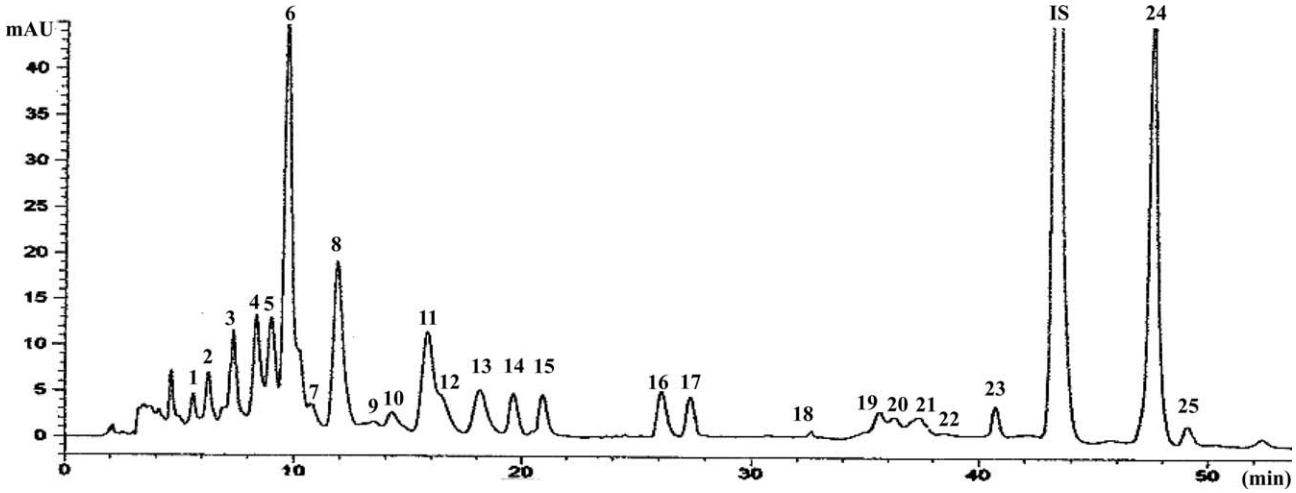

Fig. 1. HPLC chromatogram of carotenoid extract from mango after freeze-drying plus soaking in 1% sodium hydrogen sulfite solution. Peaks: 1, violaxanthin; 2, neoxanthin; 3, neochrome; 4, neoxanthin; 5, neochrome; 6, violaxanthin; 7, *cis*-neoxanthin; 8, neochrome; 9, luteoxanthin 10, *cis*-violaxanthin; 11, *cis*-violaxanthin; 12, *cis*-violaxanthin; 13, luteoxanthin ; 14 luteoxanthin; 15, luteoxanthin; 16, zeaxanthin; 17, *cis*-zeaxanthin; 18, *cis*-zeaxanthin; 19, *cis*-zeaxanthin ; 20, 9- or 9′-*cis*-lutein; 21, *cis*-β-carotene; 22, *cis*-β-carotene; 23, 15- or 15′-*cis*-β-carotene; IS, all-*trans*-α-carotene; 24, all-*trans*-β-carotene; 25, 13- or 13′-*cis*-β-carotene.

Based on the identification criteria described in the method section, dried mango was found to contain the epoxy-containing carotenoids, such as neoxanthin, violaxanthin, neochrome, luteoxanthin and their *cis* isomers. In addition, zeaxanthin and its *cis* isomers, β-carotene and its *cis* isomers as well as *cis*-lutein were also present. A similar carotenoid profile was reported by Chen et al. (2004), who determined the carotenoid composition in freeze-dried Taiwanese mango.

3.2. Content changes of carotenoids as affected by drying treatments

Table 1 shows the effect of different drying treatments on the contents of carotenoids in Taiwanese mango. Of the various carotenoids, all-*trans*-β-carotene and its *cis* isomers constituted the largest proportion (18.55 μg/g) in freeze-dried mango without soaking, followed by neochrome (8.36 μg/g), violaxanthin and its *cis* isomers (6.79 μg/g), zeaxanthin and its *cis* isomers (2.46 μg/g), luteoxanthin (2.42 μg/g), neoxanthin (1.55 μg/g), and the *cis* isomer of lutein (0.60 μg/g). Obviously, the epoxy-containing carotenoids, i.e., violaxanthin, neoxanthin, luteoxanthin and neochrome, as well as their *cis* isomers are the major carotenoids present in Taiwanese mango.

Compared to the freeze-drying treatment without soaking, the amount of violaxanthin (peak 1) decreased by 0.11 (40.7%), 0.08 (29.6%), 0.06 (22.2%) and 0.05 μg/g (18.5%) for hot-air drying (treatment A), soaked in 1% $NaHSO_3$ solution plus hot-air drying (treatment B), soaked in 1% ascorbic acid solution plus hot-air drying (treatment C) and soaked in 1% ascorbic acid solution plus freeze-drying (treatment F), respectively. This result showed that violaxanthin may undergo a significant loss under hot-air drying treatment. It was also observed that soaking in ascorbic acid solution prior to freeze-drying did not increase the yield of violaxanthin. However, an increase of 0.05 μg/g (18.5%) was found for treatment E, indicating that soaking in 1% $NaHSO_3$ before freeze-drying provided a better protective effect for violaxanthin than soaking in ascorbic acid. In addition, freeze-drying without soaking resulted in a higher yield of violaxanthin than hot-air drying without soaking. A similar trend was found for neoxanthin (peak 2), which was decreased by 0.17 (27.4%), 0.09 (14.5%) and 0.18 μg/g (29.0%), respectively, for treatments A, B and F, while an increase of 0.09 μg/g (14.5%) was shown for treatment E. However, no significant change was found between treatments C and D. Also, soaking in antioxidants, such as $NaHSO_3$ or ascorbic acid prior to drying was shown to produce a higher yield of neoxanthin than without soaking. For neochrome (peak 3), a loss of 0.90 (48.6%), 1.16 (62.7%), 0.42 (22.7%), 0.22 (11.9%) and 0.61 μg/g (33.0%), occurred for treatments A, B, C, E and F, respectively, in comparison with treatment D. However, for the hot-air drying treatment, soaking in ascorbic acid did show a higher level of neochrome than without soaking. Theoretically, neochrome may undergo oxidative loss during hot-air drying, and the increase of neochrome during hot-air drying can be attributed to conversion of neoxanthin under acidic conditions (Chen et al., 2004; Tai & Chen, 2000). However, the level of neochrome (peaks 3 and 8) in freeze-dried mango (treatment D) was found to be higher than in hot-air-dried mango (treatments A, B and C), probably because neochrome may undergo degradation as soon as it was formed from neoxanthin during hot-air drying. The same tendency was also observed for neoxanthin (peak 4), neochrome (peak 5), *cis*-neoxanthin (peak 7), *cis*-violaxanthin (peaks 10 and 11), all of which were present at the highest level for treatment E

Table 1
Contents (μg/g)[a] of carotenoids in dried mango as affected by six treatments

Peak No.	Compound	Treatment[b]					
		A	B	C	D	E	F
1	Violaxanthin	0.16[f]	0.19[e]	0.21[d]	0.27[b]	0.32[a]	0.24[c]
2	Neoxanthin	0.45[d]	0.53[c]	0.62[b]	0.62[b]	0.71[a]	0.44[d]
3	Neochrome	0.95[e]	0.69[f]	1.43[c]	1.85[a]	1.63[b]	1.24[d]
4	Neoxanthin	1.54[c]	1.72[b]	1.25[d]	0.73[e]	2.25[a]	0.73[e]
5	Neochrome	1.86[d]	2.01[c]	2.20[b]	1.50[f]	2.47[a]	1.66[e]
6	Violaxanthin	5.10[d]	8.17[b]	6.56[c]	5.01[e]	10.93[a]	4.89[f]
7	*cis*-Neoxanthin	0.19[e]	0.51[b]	0.33[d]	0.20[e]	0.57[a]	0.34[c]
8	Neochrome	4.47[d]	5.97[b]	2.55[e]	5.01[c]	7.87[a]	1.80[f]
9	Luteoxanthin	0.07[e]	0.05[f]	0.23[b]	0.08[d]	0.14[c]	0.29[a]
10	*cis*-Violaxanthin	0.30[c]	0.42[b]	0.23[d]	0.10[f]	0.48[a]	0.13[e]
11	*cis*-Violaxanthin	2.39[c]	3.32[b]	1.60[d]	0.38[e]	4.70[a]	0.39[e]
12	*cis*-Violaxanthin	0.64[d]	0.67[d]	1.01[c]	1.03[b]	1.43[a]	1.40[a]
13	Luteoxanthin	0.96[e]	1.10[b]	0.87[f]	1.24c	1.68[a]	1.46[b]
14	Luteoxanthin	0.29[e]	0.38[d]	1.98[a]	0.42[d]	1.13[c]	1.59[b]
15	Luteoxanthin	0.48[e]	0.53[e]	2.02[a]	0.68[d]	1.14[c]	1.53[b]
16	Zeaxanthin	ND	ND	ND	0.89[c]	1.15[b]	1.33[a]
17	*cis*-Zeaxanthin	0.75[d]	0.79[c]	0.79[c]	0.72[e]	0.96[a]	0.92[b]
18	*cis*-Zeaxanthin	ND	ND	0.72	ND	ND	ND
19	*cis*-Zeaxanthin	0.73[e]	0.72[e]	0.89[b]	0.85[c]	0.83[d]	0.95[a]
20	9 or 9′-*cis*-lutein	ND	0.53[c]	ND	0.60[b]	0.63[a]	0.63[a]
21	*cis*-β-Carotene	0.38[d]	0.52[b]	0.47[c]	0.35[e]	0.70[a]	0.53[b]
22	*cis*-β-Carotene	ND	0.61[a]	ND	ND	ND	ND
23	15 or 15′-*cis*-β-Carotene	0.98[e]	1.05[d]	1.47[b]	1.43[c]	1.29[c]	1.92[a]
24	all-*trans*-β-Carotene	9.32[f]	10.43[e]	13.78[d]	15.40[c]	24.68[b]	27.78[a]
25	13 or 13′-*cis*-β-Carotene	0.73[e]	1.39[c]	1.84[b]	1.37[c]	1.12[d]	3.79[a]
	Total	32.74	42.30	43.32	40.73	68.81	55.98

[a] Mean of triplicate analyses.
[b] Treatment: A, mango were dried under hot air; B, mango were soaked in 1% $NaHSO_3$ prior to hot-air-drying; C, mango were soaked in 1% ascorbic acid prior to hot-air-drying; D, mango were freeze-dried; E, mango were soaked in 1% $NaHSO_3$ prior to freeze-drying; F, mango were soaked in 1% ascorbic acid prior to freeze-drying. Each value of means bearing different letters within the same row is significantly different ($p < 0.05$).

and lowest for treatment D. This result implied that soaking in $NaHSO_3$ solution prior to freeze-drying did provide a protective effect for these epoxy-containing carotenoids. Although freeze-drying alone can also protect carotenoids from oxidative loss, our result demonstrated that the pre-soaking step is important in generating a higher yield of carotenoids. It is possible that the pre-soaking step may prevent the epoxy-containing carotenoids from degradation during the subsequent extraction procedure. Violaxanthin (peak 6) showed the same phenomenon, with the exception that treatment F generated a lower level than treatment D by 0.12 μg/g (2.4%). For neochrome (peak 8), the levels detected after treatments A, C and F were found to be lower than the treatment D by 0.54 (10.8%), 2.46 (49.1%) and 3.21 μg/g (64.1%), respectively. This outcome indicated that soaking in 1% $NaHCO_3$ solution before hot-air drying or freeze-drying could produce a higher yield of neochrome (peak 8). However, a contradictory result was shown for luteoxanthin (peak 9), with the lowest content (0.05 μg/g) for soaking in $NaHSO_3$ solution before hot-air drying. Conversely, soaking in ascorbic acid solution before hot-air drying or freeze-drying produced a higher yield than the treatment D. For *cis*-violaxanthin (peaks 10, 11 and 12), soaking in 1% $NaHSO_3$ solution was found to have a higher level than soaking in ascorbic acid solution before drying. However, for luteoxanthin (peaks 13, 14 and 15), soaking in $NaHSO_3$ or ascorbic acid solution was found to produce a higher content than the treatment D. As luteoxanthin can be formed from violaxanthin under acidic conditions, the soaking treatment may also promote formation of luteoxanthin. In addition, the antioxidant effect of $NaHSO_3$ or ascorbic acid solution may also prevent luteoxanthin loss (Tai & Chen, 2000). Zeaxanthin was detected in treatments D, E, and F, while for treatments A, B and C, it was not detected. This result clearly indicated that hot-air drying has a destructive effect on zeaxanthin. However, for freeze-drying treatment, soaking in ascorbic acid was found to result in a higher yield than soaking in $NaHSO_3$. Obviously the soaking step may prevent zeaxanthin and its *cis* isomers from oxidative loss. However, only slight difference was found for 9 or 9′-*cis*-lutein among the treatments B, D, E and F. Just like zeaxanthin and its *cis* isomers, the same trend was found for all-*trans*-β-carotene and its *cis* isomers.

By comparing the results shown above, soaking in $NaHSO_3$ prior to freeze-drying could generate the highest

yield of total carotenoids (68.81 μg/g), followed by soaking in ascorbic acid (55.98 μg/g) before freeze-drying, soaking in ascorbic acid (43.32 μg/g) or $NaHSO_3$ (42.30 μg/g) before hot-air drying, freeze-drying (40.73 μg/g) and hot-air drying (32.74 μg/g) without soaking. Among the various carotenoids quantified, all-*trans*-β-carotene plus its *cis* isomers were present in the largest amounts, followed by neochrome, violaxanthin plus its *cis* isomers, zeaxanthin plus its *cis* isomers, luteoxanthin, neoxanthin plus its *cis* isomers, and *cis*-lutein. It was also observed that treatment F produced the highest yield of all-*trans*-β-carotene, followed by treatments E, D, C, B and A. As all-*trans*-β-carotene has been demonstrated to possess high vitamin A activity and antioxidant activity (Miller et al., 1996), soaking in ascorbic acid or $NaHSO_3$ plus freeze-drying is important to protect all-*trans*-β-carotene from oxidative loss. It has been well documented that the amount of β-carotene in mango can be varied depending on cultivar, harvesting condition and maturity (Mercadante, Rodriguez-Amaya, & Britton, 1997; Mercadante & Rodriguez-Amaya, 1998). Codoy and Rodriguez-Amaya (1989) pointed out that the five cultivars of mango produced in Brazil ranged from 48–84% on a dry weight basis. Mercadante and Rodriguez-Amaya (1998) further reported that the ripe mango contains all-*trans*-β-carotene at a level of 6.7 μg/g, which equals to 17.6% of total amount of carotenoids. Our result did show a higher amount of β-carotene (15.4 μg/g) in freeze-dried mango, which can be attributed to the cultivar difference. Interestingly, no all-*trans*-lutein was detected in ripe mango. Several authors also found that all-*trans*-lutein may be converted to its epoxy-containing derivatives or *cis* isomers during ripening (Cano & de Ancos, 1994; John, Subbarayan, & Cama, 1970). In this study, we also observed the formation of 9- or 9′-*cis*-lutein from all-*trans*-lutein, and it was shown that soaking in $NaHSO_3$ or ascorbic acid prior to freeze-drying could protect 9- or 9′-*cis*-lutein from degradation. An inconsistent change was found for *cis*-zeaxanthin. This may be explained as follows: the formation of *cis*-zeaxanthin from zeaxanthin and degradation of *cis*-zeaxanthin may proceed simultaneously, and thus the dominant reaction can be dependent upon many factors, such as soaking treatment, drying temperature and time. John et al. (1970) reported that zeaxanthin constitutes a small portion (0.29%) in ripe mango, and thus it will be difficult to detect zeaxanthin in hot-air dried mango. Likewise, the inconsistent change was also found for the epoxy-containing carotenoids, such as neochrome and luteoxanthin. This is probably because that during soaking, neochrome and luteoxanthin can be formed from neoxanthin and violaxanthin, respectively. In the meantime, both neochrome and luteoxanthin may undergo isomerization or degradation simultaneously during drying. Thus, the actual yield of the individual carotenoid as affected by various drying treatments would be difficult to assess. Instead, when the total amount of carotenoid was taken into consideration, a clear trend can then be observed.

Table 2
Hunter color values of dried mango as influenced by soaking in different antioxidants

Treatment	Color[a]		
	L	*a*	*b*
Hot-air-drying			
Without soaking	67.68[b]	19.42[a]	65.97[a]
Soaked in 1% $NaHSO_3$	66.79[b]	18.71[a]	64.08[ba]
Soaked in 1% ascorbic acid	66.96[b]	18.40[a]	68.98[a]
Freeze-drying			
Without soaking	70.13[b]	10.78[b]	57.61[bc]
Soaked in 1% $NaHSO_3$	76.81[a]	7.86[c]	51.45[cd]
Soaked in 1% ascorbic acid	77.67[a]	7.28[c]	47.65[d]

[a] Each value of means bearing different letters within the same column is significantly different ($p < 0.05$).

Table 2 shows the Hunter color values of dried mango as affected by different drying and soaking treatments. With hot-air drying, no significant difference was found between soaking and non-soaking treatments, and a deep-orange appearance was observed for dried mango. However, with freeze-drying, mango without soaking treatments exhibited higher *a* and *b* values than with soaking treatment, and a yellow color was shown for both treatments. In comparison, hot-air drying could generate a darker appearance of mango than freeze-drying, probably because the browning reaction can be accelerated by the former treatment. In contrast, freeze-drying was performed at low temperature and a pale yellow color of mango was produced, which can be due to retardation of browning reaction. Nevertheless, from the commercial point of view, soaking in $NaHSO_3$ or ascorbic acid before hot-air drying can produce desirable color of dried mango and a high yield of carotenoids can still be maintained.

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