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Effect of Storage on β-Carotene Content in Mango var. *Chokanan* Puree

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ABSTRACT

This study aimed to determine the effect of storage on the content of β-carotene in mango puree. Mango (*Mangifera indica* L.) var. *Chokanan* was selected for this study. The β-carotene content at 0, 3, 6 and 24 days of storage were 4.74 ± 0.29, 3.78 ± 0.21, 3.42 ± 0.11 and 2.84 ± 0.55 mg/100 g puree, respectively. β-Carotene content of mango puree was significantly different ($P < 0.05$) at different storage times. However, a *post-hoc* test showed that the β-carotene content was significantly different ($P < 0.05$) between day 0 and 24 of storage times. Storage at 5°C for more than 3 days reduced 20% of β-carotene content in mango puree. Prolong storage time of the puree for more than 24 days had reduced about 40% of β-carotene content in mango puree. The study indicated that β-carotene content in mango puree was significantly lost after 24 days of storage.

Keywords: carotenoid, *Mangifera indica*, stability

INTRODUCTION

Nowadays, mango is one of the most significant fruits being sold on the global market. Mango fruit is normally consumed at different stages of ripening (Vasquez-Caicedo *et al*. 2005). A variety of products can be produced from its unripe and ripe forms. Pickle, chutney, mango leather, concentrate, cereal-fruit flake, fruit bar and mango powder can be made from the unripe mango. While for ripe mango, it can be processed into puree, concentrate, juice, nectar, mango blends and slices. Mango puree has been known to be the best intermediary product of mango-based products. It has been widely used for the production of beverages and leather (Schieber *et al*. 2000).

The local Malay name for mango is *mangga* or *mempelam*. Its scientific name is *Mangifera indica* Linn. and belongs to the family Anacardiaceae. Mango has a delicious taste, is high in carotenoids, vitamins A, B_2, B_3 and C, and ascorbic and folic acids (FAMA 2006). In Malaysia, four varieties of mango are mainly recommended for cultivation, namely *Golek*, *MAHA 65*, *Masmuda* and *Chokanan* (FAMA 2006). Ripe mangoes generally have a higher provitamin A content than papayas which is more than 100 μg retinol equivalent/100 g puree (Englberger *et al* 2003). β-carotene is the major carotenoid in mango and which accounts for 40 to 80% of the total carotenoids in mango (Schieber *et al*. 2000; Chen *et al*. 2007). The carotenoid composition in mango can be affected by many factors such as growth conditions, maturity, cultivar, geographical origin, degree of maturity and processing conditions (Chen *et al.* 2004, 2007). Carotenoids are prone to oxidative degradation and losses during the processing and storage of foods (Rodriguez-Amaya *et al*. 2006).

There is evidence showing that individuals with low carotenoid intake and/or low carotenoid blood levels have an increased risk of degenerative diseases (Burns *et al*. 2003). In some developing countries, dietary deficiency of vitamin A can lead to blindness and premature childhood mortality (Burns *et al*. 2003). In a number of blindness and premature childhood mortality, free radicals are thought to play a role in pathophysiology (Moore 2003). It is estimated that 254 million pre-school-aged children around the world are suffering clinical and subclinical vitamin A deficiencies (Kidmose *et al*. 2006). Carotenoids play important roles in various biological properties of animal and plants such as antioxidant activity, cell communication, immune function enhancement, UV skin protection, accessory pigments for light harvesting and free radical scavengers (Burns *et al*. 2003). They also function as quenchers of singlet oxygen, in gene activation, and in inflammation and immune processes as a modulator of lipoxygenases (Setiawan *et al*. 2001). They can alter cancer pathogenesis and have protective effects against degenerative diseases such as coronary heart disease (Rodriguez-Amaya *et al*. 2006). High intake of β-carotene is associated with lowered risk of developing lung cancer (Burns *et al*. 2003). α- and β-carotene and β-cryptoxanthin have pro-vitamin A activity which can be converted by mammals to retinol (Burns *et al*. 2003).

To date, there is still scarce information on the effect of storage times on β-carotene content of mango puree. Therefore this study was aimed to determine the effect of storage on the content of β-carotene in mango var. *Chokanan* puree.

Fig. 1 Mature and ripe mango fruit from *Chokanan* variety.

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MATERIALS AND METHODS

Sampling

In this study, a total of 2 kg mango was purchased from hypermarkets located at Wilayah Persekutuan, Putrajaya, Malaysia. The purchased mangoes were var. *Chokanan*. Mangoes that were mature and ripe (**Fig. 1**) were selected for analysis.

Chemicals

Hexane, ethanol, acetone, methanol, acetonitrile, triethylamine were from Fisher Scientific UK Limited (Bishop, UK). Toluene, potassium hydroxide (KOH), sodium sulfate anhydrous (Na_2SO_4), ammonium acetate krist were from Merck KGaA (Darmstadt, Germany). Other chemicals were purchased from Sigma Chemical Co. (St. Louis, USA). All chemicals were analytical and HPLC grades otherwise stated.

Preparation of the sample

The mangoes were washed under running tap water. Skin and seed were removed from the pulp. Then, the pulps were cut into pieces, blended and homogenized into puree using a juicer (National Juicer model MJ-C90N, Matsushita Electric Industrial Co., Osaka, Japan). About 50 g of the puree was packed into each polyethylene air-packed containers which were then flushed with nitrogen gas and sealed. The packed mango purees were stored at 5°C, and β-carotene was determined at day 0, 3, 6, 12 and 24).

Determination of β-carotene content using high performance liquid chromatography (HPLC)

Extraction of β-carotene

The extraction of β-carotene in mango puree was done according to the method of Chen *et al.* (2004) with slight modifications. Mango puree (approximate 1.0 g) was mixed with 30 ml of hexane-ethanol-acetone-toluene (10:6:7:7, v/v/v/v). Then, the mixture was agitated for 1 h in an orbital shaker (10100T, Unimax, Kelheim, Germany) at 120 rpm. The mixture was then added with 2 ml of 40% methanolic potassium hydroxide and left to saponify in the dark at room temperature for about 16 h. About 30 ml hexane was added to the mixture. After shaking for 1 min, 10% sodium sulfate solution (Na_2SO_4) was added to the volume (250 ml). After 1 min, the upper layer was collected into a round-bottom flask (500 ml) and the lower layer was repeatedly extracted twice. All the collected extracts were evaporated to dryness using a rotary evaporator (Buchi Rotavapor R-200, Buchi, Postfach, Switzerland) with a heating bath (B-490, Buchi, Switzerland) at 35°C. All of the extraction procedures were conducted in a dark room under a red dimmed light. Next, the residue of carotenoids extract in the round bottle was dissolved in 1.0 ml of methanol-acetonitrile (9:1, v/v) (0.05 M ammonium acetate, 0.1% triethylamine) before HPLC analysis.

Determination of β-carotene

The determination of carotenoids in mango puree was carried out according to the method of *Chen et al*. (2004) with slight modifications. The carotenoid extract was filtered through a 0.22 µm nylon membrane syringe filter into a brown amber bottle. The separation of β-carotene was done using a RP-C18 (250 mm × 4.6 mm I.D) stainless steel column (Zorbax Eclipse® model XDB-C18, Agilent Technologies, Palo Alto, USA) on a HPLC system, series 1100 (Agilent HPLC Model G1313A, Agilent Technologies, Palo Alto, USA) equipped with a degasser, quaternary pump, auto sampler and diode array detector. Reversed phase chromatography separation was used. Chromatography separations were done isocratically using methanol-acetonitrile (9:1, v/v) (0.05 M ammonium acetate, 0.1% triethylamine) mobile phase. Extracts (20 µl) and standards were injected separately into the HPLC. The detection wavelength used was 450 nm and flow rate was set at 1.0 ml/min and operated at 21°C.

Quantification of β-carotene contents

A known amount of standard prepared with serial concentration was used to compare the retention time of individual peaks. The quantification of β-carotene was done on the basis of linear calibration curves. *Trans*-β-carotene standard type 2 (synthetic, >95%) (Sigma Chemical, Co. St. Louis, USA) was used as an external standard. The actual β-carotene peak in the sample was directly identified by comparing with the retention time of standard. The β-carotene content in the sample was calculated from the mean of two determinations and expressed as mg per 100 g puree.

Statistical analysis

Data were expressed as means ± standard deviation of two replications. One-way ANOVA (SPSS version 13.0) and Tukey's *post-hoc* test were used to determine the mean differences of β-carotene in mango puree at different storage times. Pearson's correlation test was used to determine the correlation between the β-carotene contents and different storage times. The value of $P < 0.05$ was considered to be statistically significant.

RESULTS

Qualitative changes of mango puree

Samples consisted of 100% mango puree which was prepared from fresh and ripe mangoes in the laboratory. The freshly prepared mango puree was light orange in colour and had a sweet smell. The mango puree colour and smell were subjectively determined prior to analysis at day 0, 3, 6, 12 and 24 of storage. **Fig. 2** shows the colour changes in mango puree during storage. The changes of mango puree colour occurred gradually throughout the storage period. The colour changed from light orange at day 0 to brown at

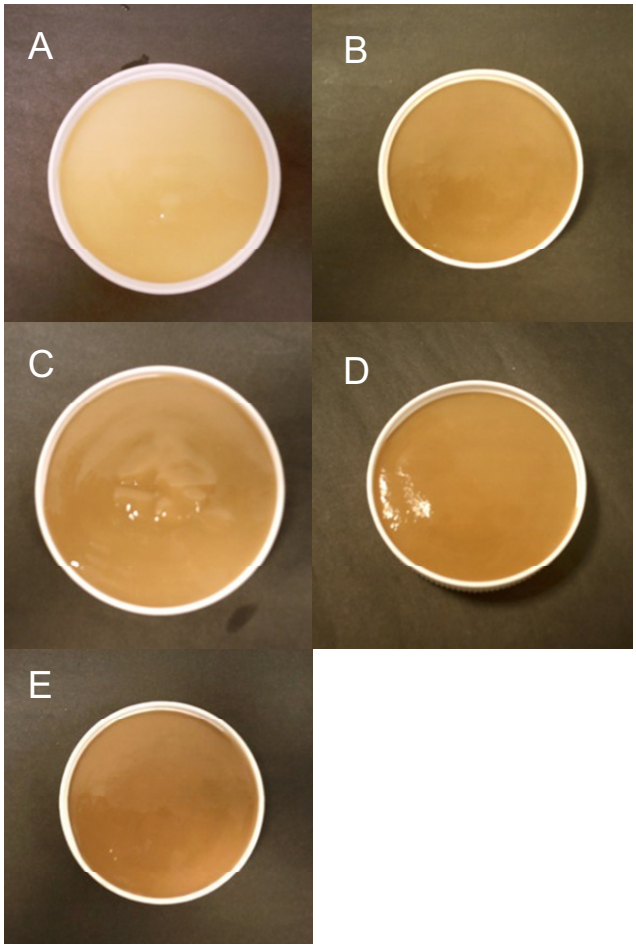

Fig. 2 Colour changes in mango puree throughout storage times at 5°C. (**A**) Day 0, (**B**) Day 3, (**C**) Day 6, (**D**) Day 12, (**E**) Day 24

Table 1 The content and percentage losses of β-carotene at different storage times.

Storage time (days)	β-carotene content (mg/100 g mango puree)	Percentage of β-carotene loss (%)
0	4.74 ± 0.29*	0*
3	3.78 ± 0.21	20.2 ± 6.12
6	3.42 ± 0.11	27.8 ± 5.56
24	2.84 ± 0.55*	40.0 ± 19.37*

Notes: Values are expressed as mean ± standard deviation of two determinations. Asterisk (*) indicate that the values are significantly different at $P < 0.05$.

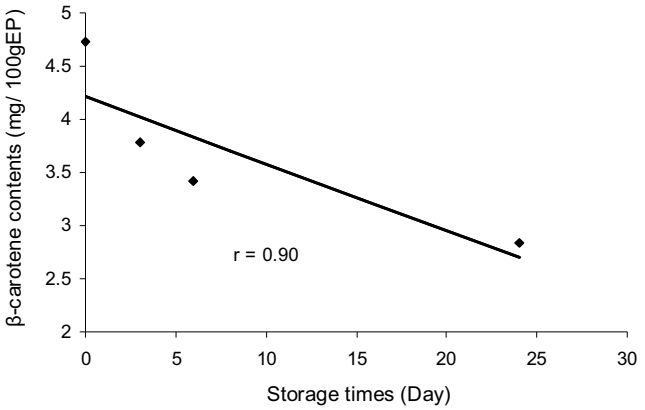

Fig. 3 Correlation between β-carotene content and storage time. The correlation was significant at $P < 0.01$.

day 24 of storage. In terms of smell, the mango puree began to emit an unpleasant smell at day 6 of storage and gradually worsened after that. The unacceptable smell was observed after 24 days of storage. The mango puree was judged to be suitable for consumption until day 6 of storage.

Quantitative changes of mango puree

The β-carotene content was in the range of 4.74 ± 0.29 to 2.84 ± 0.55 mg/100 g puree at day 0 to 24 respectively (**Table 1**). At day 0, β-carotene content was highest in mango puree (4.74 ± 0.29 mg/100 g puree) followed by day 3 (3.78 ± 0.21 mg/100 g puree), day 6 (3.42.1 ± 0.11 mg/100 g puree) then day 24 (2.84 ± 0.55 mg/100 g puree). **Table 1** also shows the percentage loss of β-carotene content in mango puree during storage. At day 3 of storage, the loss of β-carotene content was 20% followed by 28% (day 6), and 40% (day 24). A correlation between β-carotene content and storage times was done using Pearson's correlation test. **Fig. 3** shows the correlation between the two variables. There was a negative and significantly high correlation ($r = -0.90$; $P < 0.01$) between β-carotene content and storage time.

DISCUSSION

Previous studies have shown that mango is one of the major sources of carotenoids (Chen *et al*. 1996; Moore 2003; Chen *et al*. 2004, 2007). β-Carotene is the major carotenoid found in fresh mango which possesses about 40 to 80% of total carotenoids content. β-Carotene was reported to have high vitamin A activity and antioxidant capacity either in fresh mango or its processed products (Moore 2003; Chen *et al*. 2004, 2007).

Besides being consumed in a fresh form, mango is also processed into a variety of food products. Mango puree is one of the products that can be commercialized as an intermediate for juice making, nectars and the production of other beverages. However, the stability of its nutrients, especially carotenoids, as affected by storage time is poorly studied. More studies are needed to be carried out on various treatments to preserve the nutrients and stability of carotenoids to make mango puree products be more attractive and acceptable (Ciňar 2004; Chen *et al*. 2007).

Based on one-way ANOVA, β-carotene content in each sample of mango puree was significantly different over the storage period (days 0, 3, 6 and 24) at $P < 0.05$. However, when further analyzed using a *post-hoc* test, the results showed that the β-carotene content was significantly different between day 0 and day 24 of storage. It indicates that the loss of β-carotene content was significantly different after 24 days of storage.

In a study by Gil *et al.* (2006), the loss of β-carotene content in fresh-cut mango after 9 days of storage was about 12%. In the present study, the loss of β-carotene content was high at 20% on day 3 followed by 28%, and 40% on day 6 and 24, respectively. A higher loss of β-carotene observed in this study could be due to the complete destruction of cell walls and cellular membranes integrity which allowed the enzymatic oxidation (Martinez and Whitaker 1995).

In terms of its colour and smell, the former changed from light orange at day 0 to brown at day 24 of storage, while mango puree started to give an unpleasant smell at day 6. The changes in the colour of mango puree could be due to enzymatic browning caused by polyphenol oxidases as reported by Guerrero-Beltran *et al.* (2005) and Gil *et al.* (2006) in their studies on fresh-cut fruits.

During storage, most of the carotenoids are in all-*trans* form, which are highly unsaturated molecules. These molecules such as xantophylls and all-*trans*-β-carotene may undergo isomerization to their *cis*-isomers such as 9- and 13-*cis*-β-carotene. The *cis*-isomers will decrease the colour intensity, and further lead to degradation through oxidation of all-*trans* carotenoids (Bonnie and Choo 1999; Tang and Chen 2000; Ciňar 2004). This could be the way all-*trans* carotenoids are degraded during storage.

When compared with α-carotene and lutein, β-carotene is more susceptible to oxidative degradation (Tang and Chen 2000). Moreover, β-carotene possesses more conjugated carbon-carbon double bonds which make it more reactive than α-carotene and lutein. For all-*trans*-β-carotene, it can be isomerised to 9-, 13- and 15- *cis*-β-carotene during storage (Chen *et al*. 1996; Tang and Chen 2000). Dutta *et al.* (2005) also reported that the reduction of β-carotene content could be due to enzymatic oxidation and isomerization of *trans* β-carotene to a *cis*-isomer form.

There are several factors that can lead to the loss of carotenoids in mango puree. The major causes are isomerization and oxidative degradation of structure and bioactivity of carotenoids in mango due to light, the presence of oxygen and peroxides, high temperature storage, type of packaging used and duration of storage (Bonnie and Choo 1999; Moore 2003). Accoding to Dutta *et al.* (2005), lipooxygenase and other enzyme activities and coupled oxidation with lipids also promote the degradation of carotenoids. The exclusion of oxygen, minimized exposure to the light and storage at low temperature under 5°C will diminish the loss of carotenoids. In the present study, the elements of light, oxygen, temperature and type of packaging were controlled. These minimized the loss of β-carotene in mango puree during 3, 6 and 24 days of storage. In a study by Chen *et al.* (1996) on isomerization and oxidative degradation of carotenoids in carrot, it was reported that the rate of β-carotene isomerization to *cis*-isomers was greater under light storage. Besides isomerization, light also induced the oxidation to carotenoids (Chen *et al*. 1996; Bonnie and Choo 1999).

Therefore, in order to minimize the loss of β-carotene in mango puree through exposure to light, it is best to conduct the various steps of analysis in a dark room under a red dimmed light, beginning from the preparation of puree, saponification, extraction of carotenoids and the determination of β-carotene content in mango puree using HPLC.

CONCLUSIONS

The β-carotene content of mango puree stored at 5°C decreased during storage. Throughout the storage period, the

colour of the mango puree also changed gradually from light orange at day 0 to brown at the end of day 24 of storage. The mango puree started to emit an unpleasant smell at day 6. The β-carotene content was not significantly different among the storage times except for β-carotene content at days 0 and 24. This study showed that storage at 5°C for more than 3 days reduced the β-carotene content by 20%, while storage for more than 24 days resulted in further reduction in the β-carotene content to 40%. In conclusion, β-carotene content in mango puree was significantly lost after 24 days of storage.

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