Influence of Collapsed Structure on β-carotene Stability in Freeze-Dried Mangoes
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ABSTRACT
β-carotene gives benefits to human health; however, it is unstable and its degradation is accelerated by oxygen exposure. This study aimed to investigate the effects of collapsed and non-collapsed structures of freeze-dried mangoes on the stability of β-carotene. Freeze-dried mango structures were manipulated using three freeze-drying protocols. β-carotene content during storage was constantly monitored using high-performance liquid chromatography. The β-carotene degradation followed first order kinetics. The results showed that structural collapse decreased β-carotene degradation rate because the dense structures formed prevented oxygen penetration through the solids. However, the occurrence of surface cracking together with collapsed structure showed no protective effect on β-carotene. Such structure showed similar rate constant to non-collapsed systems. The cracking on mango surface has a potential to allow high oxygen penetration through the solids thus promoting β-carotene loss. These findings give benefit to the production of freeze-dried fruits to increase storage stability of β-carotene.

Keywords: Structure; Collapse; Freeze drying; Mango; β-carotene

INTRODUCTION
Mango (Mangifera indica) is a prominent tropical fruit which is widely grown in Thailand. It contains high amounts of β-carotene, a well-known carotenoid, which is responsible for the typical yellow color of the mangoes. Moreover, β-carotene is very beneficial for human consumption as it is a provitamin A and antioxidant. Among other carotenoids, β-carotene provides the highest vitamin A activity [1]. However, the high degree of unsaturated hydrocarbons makes β-carotene sensitive to oxygen exposure and its degradation mainly occurs via oxidation.

Freeze-drying produces high quality food of good appearance with well-retained flavor and nutritional quality. A successful freeze-drying process retains the volume of the material which, in the dry state, is usually highly porous, brittle and hygroscopic, but has excellent rehydration properties [2]. A typical freeze-drying process consists of three stages: freezing, primary drying, and secondary drying. Freezing of food is efficient to solidify most of the solvent which is typically water. Primary drying, or ice sublimation, begins whenever the chamber pressure is reduced and the shelf temperature is raised as to supply the heat for ice removal. Secondary drying is the stage where water is desorbed from the freeze concentrated matrix, usually at both an elevated temperature and low pressure [3].

The porous structure formed during freeze-drying is of interest for the production of food powders, instant foods, or additives because of the improved and quick rehydration properties of freeze-dried ingredients [4, 5]. However, the porous structure is not beneficial during long-term storage of freeze-dried products which contain bioactive compounds. Such compounds are affected by deteriorative reactions depending on their exposure to oxygen [5]. The oxidative stability of freeze-dried products is also reduced due to their porous structure. Porosity increases the contact area and potential exposure to oxygen [6]. Petzold & Aguilera [7] also stated that larger pores retained more occluded air in the matrix and the possibility of oxidation reactions to occur in the final product was increased.

Krokida et al. [8] stated that the physical properties of freeze-dried materials depend upon the temperature applied during freeze-drying. The aforementioned study further established the collapse of a plant material after freeze-drying above its glass transition temperature (Tg). In the freeze-drying process, the removal of ice by sublimation creates pores, the walls of which may collapse because either of the force of the surfaces or gravity. The viscosity of the freeze-concentrated matrix, which is usually the major component of the pore wall, prevents or retards collapse [9].

Collapse in freeze-dried matrices adversely affects properties of the freeze-dried materials. However, some recent studies showed that collapse can reduce the rate of some chemical reactions, such as glucose loss via Maillard browning [10], browning in lactose and lactose-protein matrices [11], lipid oxidation [12-15], and
Thermal transitions of mangoes were determined with a differential scanning calorimeter (DSC). Mangoes were purchased from Chantaburi, Thailand. They were selected for their homogeneity in size, weight, peel color, and floatation in a 4-5% NaCl solution. The total soluble solid content of mangoes was determined to be in the range of 16–20 Brix.

MATERIALS & METHODS

Raw material and chemicals
Ripe mangoes (cultivar ‘Nam Dok Mai”) were purchased from Chantaburi, Thailand. They were selected for their homogeneity in size, weight, peel color, and floatation in a 4-5% NaCl solution. The total soluble solid content of mangoes was determined to be in the range of 16–20 Brix.

Characterization of freeze-dried mango structures
The internal structures of freeze-dried mangoes were characterized with the application of a scanning electron microscope (SEM) (JEOL JSM-6480LV, Tokyo, Japan). Cross-sectioned samples were affixed on Leica Microsystems® (Switzerland) at a low magnification (10×).

Preparation of freeze-dried mango structure
Mangoes were washed, peeled, and cut into 1-cm cubes and freeze-dried (Freeze dryer Dura-Top/ Dura Stop MP, Dura Dry MP FTS Systems™, Stone Ridge, NY, USA) with the application of the 3 freeze-drying protocols, i.e., various freezing methods and freeze-drying temperatures (primary drying). The protocol 1 and 2 consisted of freezing at a shelf temperature of -35 °C, whereas mango cubes in protocol 3 were frozen by immersion in liquid nitrogen prior to transfer to the freeze-dryer at a shelf temperature of -35 °C. Frozen cubes of mangoes were kept at -35 °C for 120 min for ice formation. Then the mango samples were freeze-dried using shelf temperatures of -40 °C (protocol 1 and 3) or -15 °C (protocol 2) (primary drying), with the final product temperature (secondary drying temperature) set as 30 °C for all protocols. All freeze-dried samples were stored in evacuated desiccators over silica gel to reduce water uptake for 3 days.

Thermal transitions of mangoes
Thermal transitions of mangoes were determined with a differential scanning calorimeter (DSC). Mango juice was extracted and determined for thermal transitions as described by Lowithun and Charoenrein [18]. Mango pulp (500 g) was blended and centrifuged at 4,500g for the duration of 15 min. The supernatant was taken as mango juice. Sample juice (10-13 mg) was transferred into aluminium DSC pans and hermetically sealed. Duplicate samples were scanned without annealing to locate apparent Tg and onset of ice melting (Tm) by DSC (Perkin Elmer Pyris 1 DSC). Samples were cooled from 25 to -60 °C, held for 15 min, and subsequently heated to 25 °C at 5 °C/min. The Tg and Tm were measured after isothermal annealing at Tm-1°C obtained from the first scan. Triplicate samples were scanned from 25 to -60 °C, held for 15 min, subsequently heated to Tm-1 at 5 °C/min and held for 30 min. After that, samples were cooled to -60 °C, held for 15 min and heated to 25 °C at 5 °C/min. Tg values were taken from the midpoint of the Cm change over the glass transition and Tm from the onset temperature of the ice melting endotherm.

Freeze-dried mangoes were stored at 11.3% and 22.5% relative vapor pressure (RVP) in desiccators over saturated solutions of LiCl or CH3COOK at room temperature (25 ± 2 °C). Samples were powdered in an aluminum foil pouch to prevent uptake of water. Duplicates samples were prepared from freeze-dried materials to determine β-carotene contents with a HPLC during storage. β-carotene was extracted and measured with a method adapted from Ferruzzi et al. [19]. Freeze-dried mango powder (1 g) was extracted with 10 mL of methanol and hexane (1:1) with a homogenizer (IKA T10 basic, Ultra Turrax, Germany) for 2
min. The suspensions were centrifuged (Sorvall RC 5C Plus Superspeed Centrifuge, MN, U.S.A.) at 4,500 g at 4°C for 10 min. The supernatant (hexane layer) was collected and the precipitant was re-extracted. The collected hexane layer was dried by purging with N₂ gas. Dried residues were diluted with 2 mL of hexane prior to preparation into an HPLC amber vial to assess the β-carotene content. Each sample (20 µL) was analysed in an HPLC column C₁₈ (XTerra® RP18, 5µm, 3.9 x 150 mm) (Waters, Milford, MA, USA). Methanol:acetonitrile (9:1) with 0.1% trimethylamine was used as a mobile phase at a flow rate of 1.5 mL/min. The β-carotene was analyzed using a UV diode-array detector (Model 600, Waters, Milford, MA, USA) at a wavelength of 450 nm.

RESULTS & DISCUSSION

3.1 Thermal transition of mangoes
Mango juice samples showed two endothermic transitions prior to ice melting peak during the DSC scan which were typical of sugar-containing materials [20]. The two transitions were interpreted in different ways; however, the lower transition (T'�褡) was the glass transition of the freeze-concentrated solids phase; T'ₚ was obtained after annealing to obtain maximum freeze-concentration of solids. The second, higher temperature transition (Tₘ), showed the onset of ice melting. Mangoes contain high amounts of small molecular weight sugars that are sucrose, glucose, and fructose which gave the low T'ₛ of -53.3 °C. The Tₘ was at -38.0 °C. These thermal transitions, particularly the Tₘ, controls ice melting, directly affect the freeze-dried product structure.

3.2 Influence of freeze-drying protocols on the freeze-dried mango structure
Figure 1 shows the external structures of freeze-dried mangoes. The collapsed structure was observed in freeze-dried mangoes when frozen at -35 °C and freeze-dried at the shelf temperature of -15 °C (protocol 2) and when frozen with liquid nitrogen and freeze-dried using the shelf temperature of -40 °C (protocol 3); freezing at -35 °C followed by freeze-drying at the shelf temperature of -40 °C (protocol 1) gave non-collapsed structure.

Figure 1. Stereomicrographs of freeze-dried mango surface using freeze-drying protocol 1: freezing at -35°C and shelf temperature of -40°C (A), protocol 2: freezing at -35°C and shelf temperature of -15°C (B) and protocol 3: freezing by immersion in liquid nitrogen and shelf temperature of -40°C (C).

Mangoes freeze-dried by the application of protocol 1 underwent the freezing stages at temperatures higher than T'ₛ (-53.3 °C) but drying at a temperature lower than Tₘ (-38.0 °C). This observation was coincident with the non-collapsed structures of freeze-dried mangoes (shelf temperature -40°C). The loss of product structure or collapse during freeze drying typically occurs when the temperature of the subliming interface is maintained above the “collapse temperature” [21] which is related to the Tₘ. Sacha & Nail [22], with the aid of a freeze-dry microscope, observed that the onset of structural collapse in frozen sugar solutions was associated with the higher transition temperature or Tₘ. The result shows that the sample temperature exceeded the T'ₛ; however, it was below that of Tₘ, thus no structural collapse occurred. Moreover, the strength of mango cell walls and polymers also supported the physical structure [23]. The external structures of the samples are shown in Figure 1A. The application of freeze-drying protocol 1 was proven successful for the retention of the tissue structures of the mango samples.

The freeze-drying protocol 2 resulted in collapsed structures (Figure 1B), which was due to the melting of the ice during freeze-drying. The ice formed in the samples during the freezing stage at -35 °C. Freeze-drying was controlled by the shelf temperature of -15 °C, which was above the Tₘ. The ice could consequently melt, and the samples become dried by evaporation from a partially freeze-concentrated state rather than by
sublimation which could result in the loss of structure [21]. In addition, food matrices are plasticized by unfrozen water, thus the viscosity of the freeze concentrated matrix was decreased. This was responsible for the acceleration of the collapse during freeze-drying [9]. In addition, the result showed that the duration of the drying stage of the freeze-drying protocol 2 exceeded that of protocol 1.

Mangoes frozen by liquid nitrogen prior to freeze drying at the shelf temperature of -40 °C (protocol 3) resulted in collapsed structures, although the freeze-drying temperature of protocol 1 and 3 were identical (-40 °C). This was an evident indication of the negative effects of liquid nitrogen freezing on the structural consistency of mangoes. The result showed that after freezing in liquid nitrogen, the sample temperature increased during the freezing stage in the freeze dryer to exceed \( T'_{g} \). Freezing in liquid nitrogen produced small ice crystals and fine porosity after initial stages of the freeze-drying. This presumably increased resistance for vapor removal and a higher sublimation temperature at the sublimation interface. In addition, the liquid nitrogen freezing resulted in formation of a crust at the mango surface, which further prevented the mass transfer during sublimation. Furthermore, the cracking on surface was observed in samples which were frozen in liquid nitrogen (Figure 1C). When the internal portion of the water underwent phase transition, it caused an internal stress as a result of expansion to the surface and once the resistance of the mango was overcome, cracking of the mango surface occurred [24]. Chassagne-Bercès et al. [25] also observed the cracking of apple tissue frozen by immersion in liquid nitrogen. This indicated that a too fast freezing rate provoked breakage of food surfaces.

Figure 2 shows the SEM micrographs of the internal structure of the freeze-dried mangoes. The protocol 1 rendered non-collapsed samples with porous internal structures as shown in Figure 2A. The collapse structure caused by the application of protocols 2 and 3 revealed large gaps and dense dry layer structures because the sublimation could not be achieved; the internal water vapor pressure caused melting and water was instead removed by evaporation. This unfrozen water plasticized the dry layer formed at the beginning of drying and the internal pressure contributed to formation of a large gap inside the structure (Figure 2B).

**Figure 2.** SEM images of cross-sectioned freeze-dried mangoes using freeze-drying protocol 1: freezing at -35 °C and shelf temperature of -40 °C (A), protocol 2: freezing at -35 °C and shelf temperature of -15 °C (B) and protocol 3: freezing by immersion in liquid nitrogen and shelf temperature of -40 °C (C).

### 3.3 Stability of \( \beta- \)carotene in freeze-dried mangoes

**Figure 3.** Degradation of \( \beta- \)carotene in freeze-dried mangoes as a result of different freeze-drying protocols during storage at 11% (A) and 22% (B) RVP. ♦ protocol 1: freezing at -35 °C and shelf temperature of -40 °C, ▲ protocol 2: freezing at -35 °C and shelf temperature of -15 °C and ■ protocol 3: freezing by immersion in liquid nitrogen and shelf temperature of -40 °C.

Freeze-dried mangoes which were exposed to the three diverse freeze-drying protocols were kept at 11% and 22% RVP to determine \( \beta- \)carotene stability over time (Figure 3). Generally, storage at high RVP led to
various changes in dried foods such as structural collapse and sugar crystallization. To exclude these effects, inclusive structural transformations, the storage RVP was selected in accordance to the previous work which showed that freeze-dried mangoes stored at 25 °C at 11% RVP were in the glassy state, whereas they became rubbery at 22% RVP. However, no structural change including collapse was observed, thus both RVP were selected for monitoring the stability of β-carotene in freeze-dried mangoes during storage.

β-carotene is naturally unstable in the presence of oxygen and light. After six weeks of storage, β-carotene in samples freeze-dried by the application of protocols 1, 2, and 3 decreased approximately 57%, 43%, and 59% respectively. The amount of the degradation at both RVP was similar. β-carotene degradation followed first order kinetics which was in agreement with previous studies in both food and model systems, such as carrots [26], and a trehalose matrix [13,27].

<table>
<thead>
<tr>
<th>Freeze-drying protocols</th>
<th>Rate constant (11% RVP)</th>
<th>Rate constant (22% RVP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1473± 0.0076 a</td>
<td>0.1393± 0.0137 a</td>
</tr>
<tr>
<td>2</td>
<td>0.0907± 0.0014 b</td>
<td>0.0833± 0.0071 b</td>
</tr>
<tr>
<td>3</td>
<td>0.1418± 0.0189 a</td>
<td>0.1517± 0.0072 a</td>
</tr>
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a,b Different letters in the same column show significant differences (p≤0.05)

Rate constants of β-carotene degradation at both storage RVP are shown in Table 1. Increased rate constants indicated higher degradation rates. The results showed that the freeze-drying protocol 1 and 3 rendered similar degradation rates. In contrast, protocol 2 decreased β-carotene degradation rate significantly (P<0.05), which attributed to the collapsed structure of the freeze-dried mangoes. Structural collapse gave a dense internal matrix (Figure 2B) which has been found to increase the stability of β-carotene and other carotenoids in model systems. Prado et al. [13] observed that storage RVP at which structural collapse was found increased the stability of encapsulated β-carotene in freeze-dried polyvinylpyrrolidone. Selim et al. [14] also suggested that matrix collapse could decrease the degradation of saffron carotenoids. Similarly, Serris & Biliaderis [15] observed the lower degradation kinetics of water-soluble beetroot pigments in a collapsed matrix. Collapse of freeze-dried materials is associated with the loss of micropores and cavities through which oxygen can diffuse, so collapse of freeze-dried materials builds a barrier against oxygen penetration [14]. β-carotene is naturally more stable in the absence of oxygen. Contrarily, the higher rate constant in samples freeze-dried with protocol 1 is explained by the high porosity (Figure 2A) which allowed a higher rate of oxygen diffusion [13]. Surprisingly, structural collapse induced by liquid nitrogen renders no protection form the degenerative effects on β-carotene. It is hypothesized that the cracking of sample surface can have a potential to increase the oxygen exposure through food matrix, which results in the rapid β-carotene degradation.

CONCLUSION

Mangoes freeze-dried at temperatures above T_a had collapsed structures. Moreover, freezing by means of liquid nitrogen not only resulted in collapsed structures, but also the cracking on surface of the dried products. The results showed that collapse reduced β-carotene degradation in freeze-dried mangoes because the loss of matrix micropores and dense structure potentially prevented oxygen diffusion. The presence of cracking concurrent with collapse did not help to decrease the degradation of β-carotene. The collapse structures of freeze-dried materials give benefits on decreasing the degradation of oxygen sensitive bioactive compounds which subsequently increase the health benefits to consumers.

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REFERENCES