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COMMUNICATION

# Improving Blended Carrot-Orange Juice Quality by the Addition of Cyclodextrins During Enzymatic Clarification

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**Abstract** The purpose of this study was to evaluate the effect of cyclodextrins (CDs) on carotene content and quality of blended carrot-orange juice after enzymatic clarification. The blended carrot-orange juice was treated with 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) and gamma cyclodextrin (γ-CD) in different concentrations (1–5 g/100 mL of juice) prior to enzymatic clarification. The results showed that in non-homogenized juice added with HP-β-CD, juice acidity was generally increased from 0.83 to 1.05 g/100 mL. In the homogenized juice added with 3% (w/v) HP-β-CD, the carotene content was also increased to 5.34 mg/mL, meaning two to six times compared to control samples. However, the addition of HP-β-CD prior to juice enzymatic clarification decreased the juice clarity more than that added with γ-CD; both CDs showed wider effect on the ascorbic acid content in non-homogenized than in homogenized, when compared to control samples. The non-enzymatic browning was significantly enhanced by the addition of CDs; though, the total polyphenol content increased with the addition of HP-β-CD from 32.59 to 37.09 and from 29.20 to 35.44 mg GAE/mL in non-homogenized and homogenized juice, respectively. The juice color turned red-yellow with increase in the carotene content of the juice. In conclusion, the addition of HP-β-CD in homogenized juice improved the nutritional and some physicochemical parameters of the juice.

**Keywords** Cyclodextrin · Enzymatic clarification · Blended carrot-orange juice · Homogenized juice · Non-homogenized juice

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## Introduction

Carrot (*Daucus carota*) is an important root vegetable cultivated worldwide. The carrot juice is an appreciable source of carotene and acceptable for its vitamin and mineral contents (Di Giacomo and Taglieri 2009; Day et al. 2010). In recent years, a steady increase of carrot juice consumption has been reported in many countries. Carotene has been reported to be a free radical quencher, biological antioxidant and an anticancer compound (Filotheou et al. 2010).

Orange (*Citrus cinensis*) on the other hand is widely grown worldwide and serving as one of the most abundant sources of vitamin C. Orange juice is particularly known for its fresh flavor, and its added value as a natural source of antioxidants which are beneficial to human health (Gardner et al. 2000).

Juice blending is one of the methods used to improve the nutritional quality and sensory characteristics of the juice product. The vitamin and mineral contents in the blended juice are improved depending on the kind and quality of fruits and vegetables used (De Carvalho et al. 2007). The homogenization process is also used to improve the juice quality by reducing the large pulp particles in the juice into smaller sizes. The high pressure process induces pectin release from the middle lamella due to further breakdown of the tissues and other large molecules incorporated therein. These particles are then dispersed into a colloidal system to lower the viscosity of the juice (Sinchaipanit and Kerr 2007).

Clarification is a beneficial step in juice processing and improvement of consumer acceptability. It is often achieved through enzymatic treatment, membrane filtration, or using clarifying aids. The use of commercial pectin enzymes is common in fruit juice processing. The advantages of pectin enzyme use have been to increase the flow of juice, clarity, improve juice yield, and facilitate filtration (Alkorta et al. 1998).

Cyclodextrins form complexes with natural colors, flavors, vitamins and increases solubility of different compounds, allowing its use as a carrier and stabilizer for these additives. Hydroxypropyl beta cyclodextrin (HP-β-CD) is a modified β-CD having a higher aqueous solubility (>60%) and a proven safe profile, especially for inclusion uses. It is an alternative to α-, β- and γ-cyclodextrin, with improved water solubility properties (Sarah and Robert 2005). γ-Cyclodextrin is a cyclic polymer consisting of eight glucose units linked by α-1,4 bonds. It is produced enzymatically from liquefied starch.

The purpose of this study was to evaluate the effect of cyclodextrins on carotene content and quality of blended carrot-orange juice after enzymatic clarification.

## Materials and Methods

Carrots and oranges were purchased from the fresh fruit market *Da Runfa* in Wuxi, Jiangsu, China. Commercial pectinolytic enzyme, Pectinex Ultra SP-L from *Aspergillus aculeatus* was obtained from Novozyme, (Beijing, China). 2-Hydroxypropyl-β-Cyclodextrin, 98% (HP-β-CD) was purchased from Zibo Qianhui Fine Chemical Co., Ltd (Shandong, China) and γ-Cyclodextrin, 98% (γ-CD) was purchased from Jiangsu Fengyuan Biotechnology Co., Ltd. Other chemicals used were of analytical grade and were purchased from Shanghai Chemical Reagent Co. Ltd, China.

### Juice Extraction; 2-Hydroxypropyl-β and γ-Cyclodextrin and Enzymatic Treatment

Carrot and orange juices were prepared in proportion (70:30 v/v) according to the method of Karangwa et al. (2010) using 3 kg of carrots and 5–6 kg of oranges, respectively. The blended carrot-orange juice was divided into two parts: one part was used as non-homogenized juice and the other part was homogenized before addition of 2-Hydroxypropyl-β-cyclodextrin or γ-Cyclodextrin in different concentrations of 1–5 g/100 mL of juice. The juice was stirred for 90 min at room temperature using a magnetic stirrer to mix up the cyclodextrins. Finally, the mixture was subjected to pectin enzyme treatment for clarification following the optimum conditions (Karangwa et al. 2010).

### Juice Characterization

Control samples were non-homogenized and homogenized clear blended carrot-orange juice treated with pectin enzyme only.

*Titratable acidity* (TA) was determined according to the method of Qudsieh et al. (2001). TA was expressed as g/100 mL of citric acid. *Juice clarity* was determined according to the method of Karangwa et al. 2010 and was expressed in absorbance.

*Total carotene content* (*TCC*) measurement was carried out according the method of Liao et al. (2007). The total carotene content (mg β-carotene/mL) in the sample was calculated from the standard curve $y = 0.43x + 0.002,\ (R^2 = 0.9974)$.

*Total polyphenols content* (*TPC*) was determined by the Folin-Ciocalteu method (Singleton and Rossi 1965). The total polyphenol content (mg gallic acid equivalent (GAE)/mL) was calculated by the standard curve $y = 0.0011x + 0.012, (R^2 = 0.9985)$.

*Ascorbic acid content* (*AaC*) was determined by iodine titration method (AOAC 1999). The ascorbic acid was expressed in mg/100 mL of juice.

*Non-enzymatic browning* (*NEB*) was measured using the method of Meydev et al. (1977). The NEB was expressed in absorbance and measured direct after clarification.

*Color measurement* was evaluated according to the method of Rao et al. (2011) using color difference meter (Shanghai Precision & Scientific Instrument Co., Ltd. (Shanghai, China)) and de-ionized water as blank. Two milliliters of sample were pipetted into a 5 cm cuvette and the reflectance was measured directly from the juice sample. The mean of three values was considered to evaluate the color in the CIE-L,a,b space system. The system provides the values of three color components: the higher L (black-white component, luminosity) indicate higher lightness, and the chromaticness coordinates, a (+red to -green component) and b (+yellow to -blue component).The $\Delta E$ index was calculated from the equation: $\Delta E = \left(\Delta L^2 + \Delta a^2 + \Delta b^2\right)^{1/2}$.

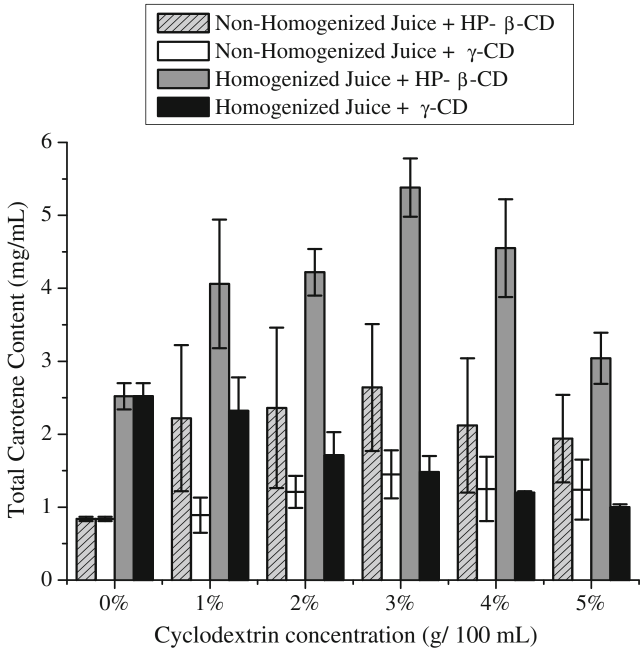


**Fig. 1** Total carotene content in function of 2-Hydroxypropyl-β-Cyclodextrin and γ-Cyclodextrin concentration in non-homogenized and homogenized clear blended carrot-orange juice

## Statistical Analysis

Analysis of variance (ANOVA) was carried out by using the software SPSS 16 (Chicago IL). Significant differences ($p<0.05$) among treatments were detected using Duncan's

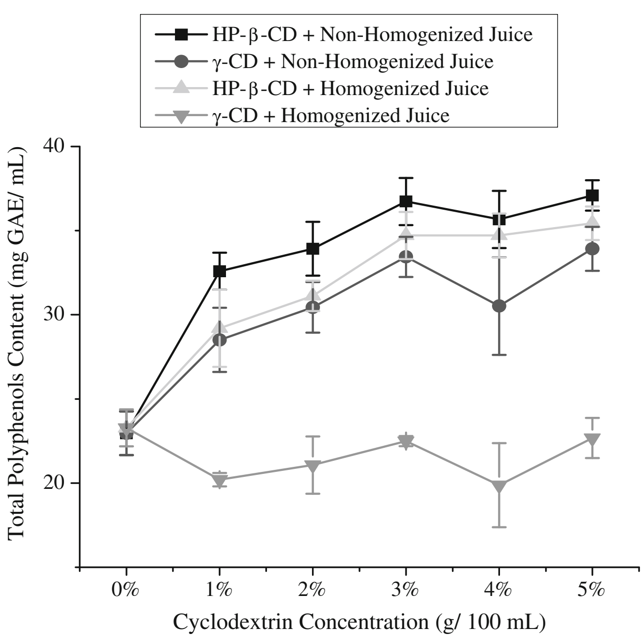


**Fig. 2** Total polyphenol content in function of 2-Hydroxypropyl-β-Cyclodextrin and γ-Cyclodextrin concentration in non-homogenized and homogenized clear blended carrot-orange juice

**Table 1** Nutritional and physicochemical parameters of clear blended carrot–orange juice treated with cyclodextrins

| | | HP-β-CD | | | | | | γ-CD | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0% | 1% | 2% | 3% | 4% | 5% | 1% | 2% | 3% | 4% | 5% |
| AaA (mg/ 100 mL) | NHJ | 17.61±0.0 | 15.41±3.1 | 17.61±0.0 | 19.81±3.1 | 15.41±3.0 | 13.21±3.1 | 11.01±3.1 | 15.41±3.0 | 19.81±2.5 | 15.41±3.1 | 15.41±3.1 |
| | HJ | 12.93±0.2 | 12.56±0.2 | 13.04±0.3 | 14.03±0.1 | 12.96±0.3 | 12.70±0.1 | 12.06±0.5 | 12.86±0.1 | 12.03±0.1 | 11.96±0.3 | 11.50±0.3 |
| Clarity | NHJ | 0.020±0.8 | 0.035±0.0 | 0.041±0.0 | 0.042±0.0 | 0.033±0.0 | 0.032±0.0 | 0.011±0.0 | 0.013±0.0 | 0.015±0.0 | 0.014±0.0 | 0.011±0.0 |
| | HJ | 0.021±0.8 | 0.046±0.0 | 0.050±0.01 | 0.062±0.01 | 0.041±0.0 | 0.029±0.0 | 0.038±0.0 | 0.035±0.0 | 0.030±0.0 | 0.027±0.0 | 0.015±0.0 |
| TA (g/100 mL) | NHJ | 0.39±0.02 | 0.84±0.0 | 0.83±0.0 | 0.87±0.0 | 0.89±0.0 | 1.05±0.0 | 0.62±0.02 | 0.71±0.01 | 0.72±0.01 | 0.71±0.05 | 0.74±0.03 |
| | HJ | 0.44±0.02 | 0.50±0.01 | 0.503±0.0 | 0.536±0.0 | 0.543±0.01 | 0.550±0.0 | 0.42±0.01 | 0.44±0.0 | 0.47±0.02 | 0.56±0.03 | 0.61±0.07 |
| NEB | NHJ | 0.045±0.002 | 0.071±0.0 | 0.064±0.0 | 0.066±0.0 | 0.066±0.0 | 0.078±0.0 | 0.083±0.01 | 0.081±0.0 | 0.081±0.0 | 0.071±0.0 | 0.067±0.0 |
| | HJ | 0.039±0.0 | 0.049±0.01 | 0.050±0.0 | 0.058±0.0 | 0.064±0.0 | 0.067±0.01 | 0.039±0.0 | 0.050±0.01 | 0.040±0.0 | 0.049±0.0 | 0.052±0.01 |

TSS: Total Soluble Solid; AaA: Ascorbic Acid Content; TA: Titratable acidity; NEB: Non-Enzymatic Browning

NHJ: Non-Homogenized Juice; HJ: Homogenized Juice

Values are means ± standard deviation of three determinations ($n=3$)

[a] 0%: Control (non-homogenized and homogenized juice treated by enzyme only)

multiple range tests. Values expressed are means ± standard deviation of triplicate measurements.

## Results and Discussion

Beta-carotene is a precursor of vitamin A and plays an important role in metabolism and human health maintenance. Carotenoids are potent biological singlet-oxygen quenching agents, having poor water solubility. Various methods have been used to increase the water solubility of carotenoids such as using cyclodextrins to form complexes with the carotenoids (Polyakov et al. 2004). There was a general increase in TCC in juice samples added with HP-β-CD and γ-CD compared to control juice samples (Fig. 1). The TCC in control non-homogenized and homogenized samples was 0.84±0.03 and 2.52±0.18 mg/mL, respectively. The increase of TCC in non-homogenized juices with HP-β-CD (2.64±0.87 mg/mL), γ-CD (1.45±0.33 mg/mL) was significant ($p<0.05$) (Fig. 1). The TCC in homogenized juices added with HP-β-CD (5.38±0.4 mg/mL) and γ-CD (2.32±0.46 mg/mL) were highly significant ($p<0.001$). The results showed that the addition of 3% (w/v) HP-β-CD into homogenized juice prior to enzymatic clarification increased the TCC two to six times compared to control samples. It is likely that homogenization reduced the particle size of the juice to release the carotenoids and in addition, it may assist CDs as complexing agents forming inclusion with carotenoids in clear juice to improve the carotene solubility.

Tsai et al. (2007) reported that pink pummelo juice had higher total polyphenol content and antioxidant potential than white pummelo juice due to pigments such as carotenoids which were higher in pink pummelo than in white pummelo. The addition of HP-β-CD and γ-CD prior to clarification of non-homogenized juice showed significant effect on TPC (Fig. 2). TPC increased significantly ($p<0.05$) from 32.59 to 37.09 mg GAE/mL and from 28.51 to 33.92 mg GAE/mL with the addition of HP-β-CD and γ-CD, respectively in non-homogenized samples. TPC in homogenized juice added with HP-β-CD also increased significantly from 29.20 to 35.44 mg GAE/mL. The addition of γ-CD to homogenized juice showed negligible change (20.2 to 22.68 mg GAE/mL) when compared to control non-homogenized and homogenized samples (22.96 and 23.28 mg GAE/mL) respectively. This increase may be attributed to the solubilization of some insoluble polyphenols in juice by formation of a soluble inclusion complex with cyclodextrin.

Analysis of Ascorbic acid content as another nutritional quality index showed slight differences after treatments. As shown in Table 1, The addition of HP-β-CD (13.21 to 19.81 mg/100 mL) and γ-CD (11.01 to 19.81 mg/100 mL) in non-homogenized juice compared with control sample (17.61 mg/100 mL) shows a wider range on AaC ($p<0.05$). In homogenized juice, the addition of HP-β-CD and γ-CD (Table 1) manifests an insignificant range (11.50 to 14.03 mg/100 mL) when compared to the control sample (12.93±0.2 mg/100 mL).

The color change in carrot juice involves the co-precipitation of color substances such as β-carotene with larger molecules or enzymatic and oxidative discoloration (Lan et al. 2005). From Table 2, $\Delta L$ (lightness) for control samples was −1.64 and −5.54 respectively. The addition of HP-β-CD and γ-CD to non-homogenized and homogenized juices decreased $\Delta L$ to −4.47; −3.79; −7.49 and −1.35, respectively. The control homogenized sample ($\Delta a$ and $\Delta b$) presented a characteristic color of red-yellow, compared to control non-homogenized sample ($\Delta a$ and $\Delta b$) presenting green-blue color. While, the $\Delta a$ and $\Delta b$ values of the samples with HP-β-CD were greater, meaning the juice turned to red and yellow in color more than those added with γ-CD. For the non-homogenized and homogenized samples added with HP-β-CD and γ-CD, the total color difference

**Table 2** The color property of the clear blended carrot-orange juice treated with cyclodextrins

| | HP-β-CD + Non-Homogenized Juice | | | | γ-CD + Non-Homogenized Juice | | | | HP-β-CD + Homogenized Juice | | | | γ-CD + Homogenized Juice | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ΔL | Δa | Δb | ΔE | ΔL | Δa | Δb | ΔE | ΔL | Δa | Δb | ΔE | ΔL | Δa | Δb | ΔE |
| 0% | −1.64 | 1.81 | 3.79 | 4.51 | −1.64 | 1.81 | 3.79 | 4.51 | −5.54 | 4.69 | 7.65 | 10.54 | −5.54 | 4.69 | 7.65 | 10.54 |
| 1% | −4.37 | 5.14 | 5.48 | 8.69 | −2.21 | 2.69 | 3.42 | 4.88 | −5.59 | 4.48 | 7.78 | 10.58 | −1.35 | −0.14 | 8.41 | 8.52 |
| 2% | −4.44 | 5.33 | 5.5 | 8.85 | −2.74 | 2.74 | 3.99 | 5.56 | −6.82 | 5.37 | 8.73 | 12.32 | −1.16 | −0.39 | 7.19 | 7.29 |
| 3% | −4.47 | 5.88 | 5.55 | 9.24 | −3.79 | 2.88 | 4.25 | 6.38 | −7.49 | 5.56 | 10.38 | 13.96 | −0.95 | −0.49 | 5.31 | 5.41 |
| 4% | −3.99 | 4.24 | 5.49 | 8.00 | −3.01 | 2.41 | 3.2 | 5.01 | −5.75 | 3.6 | 9.1 | 11.35 | −0.89 | −0.50 | 5.41 | 5.50 |
| 5% | −3.76 | 3.34 | 5.21 | 7.24 | −2.78 | 1.6 | 3.15 | 4.49 | −5.12 | 2.85 | 7.66 | 9.64 | −0.81 | −0.75 | 4.63 | 4.76 |

[a] 0%: Control (non-homogenized and homogenized juice treated by enzyme only)

($\Delta$E) increased significantly with values of 9.24, 6.38, 13.96 and 8.52, respectively compared to control samples with 4.51 and 10.54, respectively.

Non enzymatic browning reactions mainly cause color change, sugar and vitamin C loss and 5-HMF formation, affecting the quality of fruit juices (Ibarz et al. 1999). The addition of HP-β-CD in non-homogenized and homogenized samples increased NEB significantly from 0.064 to 0.078 and 0.049 to 0.067, respectively. On the other hand, the addition of γ-CD in non-homogenized showed a significant decrease of NEB from 0.083 to 0.067, while, in homogenized samples, NEB increased significantly from 0.039 to 0.052. The results showed that the addition of HP-β-CD and γ-CD prior to juice clarification enhanced the NEB in clear blended carrot-orange juice.

The increase in carotene content of the juice influences its clarity. As shown in Table 1, a great decrease in juice clarity was observed in non-homogenized and homogenized juice samples added with HP-β-CD and γ-CD compared to control juice samples. The addition of HP-β-CD in both non-homogenized and homogenized juice reduced the clarity level to 0.042 and 0.062, respectively. Addition of γ-CD to non-homogenized and homogenized juice changed the clarity level to 0.015 and 0.038, respectively, compared to control samples with a clarity level of 0.020 and 0.021, respectively. This might be explained by the fact that increases in carotene content as pigment reduces the clarity by reducing the juice transparency and makes the UV-rays difficult to pass through it.

There was a significant increase in TA in non-homogenized and homogenized samples added with HP-β-CD and γ-CD compared to control samples (Table 1). TA in control samples was 0.39 and 0.44 g/100 mL, respectively. The TA of non-homogenized juice samples with HP-β-CD and γ-CD increased from 0.83 to 1.05 g/100 mL and 0.62 to 0.74 g/100 mL, respectively. For the homogenized juice, the TA increased from 0.50 to 0.55 g/100 mL and 0.42 to 0.61 g/100 mL, after addition of HP-β-CD and γ-CD, respectively. This increase in acidity might be due to the formation of acid by degradation of polysaccharides and oxidation of reducing sugars or by breaking down of pectin substances (Hussain et al. 2008).

## Conclusion

The addition of cyclodextrins prior to enzymatic clarification provides an alternative solution to the depletion of carotene content in clear blended carrot-orange juice. The results from this study show that the addition of 3% HP-β-CD in homogenized blended carrot-orange juice improves carotene content, though reduces slightly its clarity.

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