Effects of Lipophilic Continuous Phases and Heating Methods on Thermal Degradation and Isomerization of β -Carotene

Suk Hoo Yoon*

Department of Food Science and Biotechnology, Woosuk University, Samnye, Jeonbuk 55338, Korea

Received August 11, 2015
Revised October 7, 2015
Accepted October 22, 2015
Published online February 29, 2016

*Corresponding Author
Tel: +82-63-290-1514
Fax: 82-63-291-9312
E-mail: shooyoon@woosuk.ac.kr
sukhooyoon@gmail.com

pISSN 1226-7708
eISSN 2092-6456

© KoSFoST and Springer 2016

Abstract Rates of thermal degradation and isomerization of all-*trans*- β -carotenes in air and in triacylglycerols were determined. Degradation of carotenes in triacylglycerols was faster than that in air. The 13-*cis*- β -carotene level in triacylglycerols was higher than in air. Oxidized materials of triacylglycerols probably facilitated isomerization of carotenes and, thus, degradation. Amounts of all-*trans*- β -carotenes and all-*trans*- α -carotenes in pumpkin decreased with an increase in heating time. The proportion of 13-*cis*- β -carotene increased after heat treatment started, probably due to thermal isomerization of all-*trans*- β -carotenes to *cis*-isomers, and to decreases in amounts of all-*trans*- β -carotenes and all-*trans*- α -carotenes in pumpkin. Effects of heating methods on proportions of isomers; however, were not different.

Keywords: β -carotene, thermal degradation, isomerization, triacylglycerol, pumpkin

Introduction

The carotenoids are yellowish red coloring compounds distributed widely in nature, and there were a number of reports to show prevention and curing activities of carotenoids against lung cancers and several diseases including coronary and cardiovascular diseases (1–3). It was reported that all-*trans*- β -carotene (TBC) quenched singlet oxygen (4), and acted as efficient antioxidant through removal of oxygen radicals (5,6). TBC, however, played role as prooxidant depending on the environments (7,8). Most natural α -carotenes are present in *trans* form (9), whereas the significant amounts of *cis* isomers, however, are detected in thermally processed foods, and foods stored under light (10,11).

The degradation and isomerization pattern of carotenes are affected by the environments of carotenes (12–13). The isomerization of *trans*- β -carotene to 9- and 13-*cis*- β -carotene was observed when β -carotene was dissolved in palm and coconut triacylglycerols (12), while 13- and 15-*cis*- β -carotene were produced more in olive oil (13).

Pumpkin (*Cucurbita moschata*) is a food vegetable grown and consumed globally in many regions, and pumpkin flesh and seeds have been used as food and medicinal stuffs (14). Several pumpkins, such as *C. moschata*, *C. maxima*, and *C. pepo*, possess large amount of α - and β -carotenes, and, thus, showed thick yellow and orange colors (15).

The different cooking methods affected the isomerization and degradation of carotenes in pumpkins (16). An increase in β -carotene of pumpkin was observed when boiling and stir-frying of pumpkin (16).

Blanching of vegetables is a thermal treatment frequently used in industry before freezing, since blanching can inactivate enzymes, destroy microbial cells, and maintain product quality high (17–19). Apart from blanching process, the heat sterilization is used to extend the shelf life of commercial food products by inactivation of vegetative cells and spores accompanied with nutrient loss (20,21). The aims of this study were analysis of β -carotene isomerization in air, and in oil after dissolution in triacylglycerols (TAG) as a lipophilic environment surrounding carotenes. Evaluation of degradation and isomerization of β -carotene in pumpkins due to blanching and retort sterilization was also performed.

Materials and Methods

Materials Standard materials of all-*trans*- β -carotenes (TBC) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ripe pumpkins were obtained from a local market in Seoul, Korea, a day after harvest. Refined, bleached, and deodorized (RBD) soybean oil was also purchased from a local market in Seoul, Korea. Chemicals and organic solvents used were of analytical grade unless specified otherwise. Organic solvents used for HPLC were HPLC grade purchased from Mallinckrodt Co. (Paris, KY, USA). HPLC grade solvents were filtered using a membrane filter (0.45 μ m) (Acro LC13; Gelman, Port Washington, NY, USA) followed by degassing using vacuum pump (Millivac-Mini Vacuum Pump, XF5423050; Merck KGaA, Darmstadt, Germany).

Heating of TBC in air and in purified oil Twenty mg of TBC, equivalent to 27,510 µg of a TBC standard material, was put in a glass vial covered with aluminum foil and heated in a furnace with a thermostat at 180°C for 20 min (22). TBC samples were allowed to stand in an ambient temperature to room temperature after heating, and dissolved in a mixture of hexane and acetone (6:4) prior to analysis. Twenty mg of the TBC standard material was dissolved in 3 g of purified soybean oil, and placed in a furnace heated at 180°C for 20 min. Purified soybean oil was prepared to obtain pure TAG following a previous method (23). Soybean oil was passed through a chromatographic column (60 cmx4 cm) packed with a series of 100 g of activated silicic acid (100 mesh) (Mallinckrodt Co.), 30 g of a 2:1 mixture of activated charcoal (J.T. Baker Chemical Co., Phillipsburg, NJ, USA), and 120 g of celite (Sargent Welch, Cleveland, OH, USA). Soybean oil passed through the chromatographic column was referred to as purified soybean oil.

Blanching and retort of pumpkin Pumpkins were washed with tap water, dried with a soft cloth, and the skin as peeled (16). Skinless pumpkin flesh was then cut into small pieces of 1.5 cmx1.5 cmx1.5 cm (lengthxwidthxheight), and divided into seven portions of 10 g each. One portion was maintained raw while others were heat-treated. Ten g of pumpkin flesh was boiled in water at 100°C for 3 min, then pumpkin samples were drained and cooled before an extraction process. For retort sterilization, pumpkin samples were placed in a general steam retort heated at 121°C for 1 h. After blanching and retort, pumpkin samples were cooled in an iced water bath for 2 min.

Extraction of carotenes Carotenes were extracted from the purified oil phase enriched with carotenes (24). One g of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ was added to 1 g of the oil phase and 50 mL of an extraction solution of 50% hexane, 25% acetone, 25% ethanol, and 0.1% BHT. The mixture was stirred vigorously for 20 min at 4°C using a vortex mixer, and, after addition of 15 mL of reagent grade water, the mixture was stirred for another 10 min at 4°C. The organic phase containing β -carotene was collected and filtered using Chromafil PET filters (0.20 µm pore size, 25 mm diameter) (Macherey-Nagel GmbH & Co., Düren, Germany).

Extraction of carotenes from pumpkin was carried out following a previous method (16). Five g of a pumpkin sample was subjected to extraction using 50 mL of ethanol in a 250 mL conical flask. The flask was placed in a water bath at 25°C for 1 h with regular vigorous stirring. The resulting crude extract was then filtered through a 0.4 µm filter (Whatman, Maidstone, UK), and the solvent was evaporated away under vacuum using a rotary evaporator (Rotavapor R-215; BUCHI Labortechnik AG, Flawil, Switzerland). Extracts were kept at -20°C in a deep freezer prior to analysis.

HPLC analysis and identification of carotene isomers An HPLC instrument consisting of a Jasco PU-980 pump (Jasco, Hachioji,

Japan), a Rheodyne detector (Anspec, Ann Arbor, MI, USA), a UV-975 detector set at 450 nm (Anspec), a 5 µ Vydac 201TP54 column (4.6 m x250 mm) (Anspec), and CO-965 column oven (Anspec) set at 25°C were used (22). The solvent flow rate was 0.7 mL/min and the solvent system used was solvent A, acetonitrile:methanol=3:2 (v:v); and solvent B, acetonitrile:methanol:isopropyl alcohol:chloroform:methylene chloride=3:2:5:0.5:0.5 (v:v:v:v). Solvent A flowed for 5 min, followed by solvent B in a step gradient manner. A stock solution of TBC was prepared from 15 mg of crystalline TBC in a 100 mL volumetric flask made to a volume of 100 mL with hexane and acetone (6:4, v:v). Appropriate aliquots of the solution were diluted for HPLC analysis, and the carotene content was determined using a calibration curve obtained from determination previously. Identification of individual carotenes was carried out using retention time comparisons of individual standard materials. Absorption maxima based on UV-visible spectra obtained using a V500 spectrophotometer (Jasco) were also used for identification of isomers. Individual isomers was collected using HPLC.

Statistical analysis All experimental values were expressed as mean values of triplicate determinations and standard deviation (SD) values were less than ±3% unless specified. Respective SD values were not given when $\text{SD} \leq 3\%$. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS, Chicago, IL, USA) to ascertain significant differences between treatments at $p < 0.05$.

Results and Discussion

HPLC analysis of carotenes An HPLC chromatogram of carotenes extracted from fresh pumpkin is shown in Fig. 1. Each peak was identified based on comparison of retention times of individual carotenes and quantified using a UV-visible spectrophotometer (Anspec). Elution patterns of carotenes were consistent with results previously reported (25-27). The total carotene content in fresh pumpkin was 230 ± 6.1 µg/g of wet pumpkin. Pumpkin carotenes were composed of TBC ($51.2 \pm 1.3\%$), all-*trans*- α -carotene (TAC) ($32.9 \pm 0.9\%$), and 13-*cis*- β -carotene ($15.9 \pm 0.4\%$). Trace amounts 15-*cis* and 9-*cis*- β -carotenes were also detected. The carotene content of pumpkin varied depending upon species; however, the carotene composition of pumpkins obtained was consistent with previous reports (28,29).

Thermal degradation and isomerization of TBC during heating

The total carotene content of TBC standard materials in glass vials before heating was 27,510 µg, and decreased gradually with an increase in air heating time (Fig. 2). The TBC content decreased significantly ($p < 0.05$) more rapidly in purified oil than in air. After 20 min of heating, the TBC content reached 2,500 µg in air and 600 µg in purified oil. Heating for more than 20 min caused loss of

Fig. 1. HPLC profiles of carotenes extracted from fresh pumpkin (peak A, 15-*cis*- β -carotene; peak B, all-*trans*- β -carotene; peak C, 13-*cis*- β -carotene).

Fig. 2. Effect of heating time on the total carotene content in air (●) and dissolved in oil (○). Values are means of triplicate determinations. Error bars are not shown when $SD \leq \pm 3\%$.

90% of total carotene, regardless of the environment. β -*trans*-carotene reportedly was isomerized first, then degraded into a series of apocarotenals during autooxidation (30).

As a lipophilic continuous phase surrounding carotenes, the purified soybean oil was used in this study. The purified soybean oil used was pure TAG lacking pigments, free fatty acids, and impurities. Most plant oils contain the natural anti-oxidants tocopherols that might affect isomerization and degradation of carotenes (31). In this study, the purified soybean oil used was a lipophilic continuous environment instead of soybean oil to eliminate anti and/or pro-oxidant effects of materials other than triacylglycerols (TAG). Reportedly, β -carotene promotes oxidation of corn oil TAG during heating (32). TAG oxidation products may have accelerated degradation of β -*trans*-carotene, and *vice versa*, because oxidation products of TAG had pro-oxidant activity against β -*trans*-carotene in isomerization and degradation process (33,34).

The heating time affected the residual TBC contents significantly. The TBC contents decreased exponentially (Fig. 2), and thus the semi-logarithmic plot of TBC contents depending upon heating time showed the linear lines with correlation coefficients of 0.9923 (in air), and 0.9903 (in oil) (Fig. 3). TBC decreased more rapidly in the oil than

Fig. 3. Semi-logarithmic plot of total carotene content with respect to heating time in air (●) and dissolved in oil (○). Values are means of triplicate determinations. Error bars are not shown when $SD \leq \pm 3\%$.

Fig. 4. Effect of heating time on the composition of carotene isomers in air (A) and dissolved in oil (B) (● all-*trans*- β -carotene; ○ 15-*cis*- β -carotene; ■ 13-*cis*- β -carotene; □ 9-*cis*- β -carotene). Values are means of triplicate determinations. Error bars are not shown when $SD \leq \pm 3\%$.

in the air ($p < 0.05$), and the decrease rates were -0.1705 and -0.1306 min^{-1} , respectively.

The effect of heating time on compositions of individual carotene isomers in air is shown in Fig. 4. The major isomers produced were 15-*cis*, 13-*cis*, and 9-*cis*- β -carotene (Fig. 4A). The variety of TBC isomers identified was consistent with previous reports (11,30,32, 35). Contents of 15 and 9-*cis*- β -carotene decreased continuously after heating started, whereas the content of 13-*cis*- β -carotene increased right after heating started, then significantly ($p < 0.05$)

Fig. 5. Effect of heating method on the carotene composition in pumpkin (■ total carotenes; ■ all-*trans*-β-carotene; ■ all-*trans*-α-carotene; □ 13-*cis*-β-carotene). Values are means of triplicate determinations. Error bars are not shown when $SD \leq \pm 3\%$.

decreased thereafter, compared with controls. TBC was probably isomerized to 13-*cis*-β-carotene first as an intermediate, then further degraded to other materials. An increase in the 13-*cis*-β-carotene content has also been reported in the beginning of heating at 125 and 150 (11). Results reported herein coincided with previous reports showing more production of 13-*cis*-β-carotene in the beginning of heating (9,35).

The individual carotene isomers changed significantly ($p < 0.05$) during heating, compared with controls. The proportion of TBC decreased in the first 15 min of heating, then increased thereafter ($p < 0.05$). The proportion of 13-*cis*-β-carotene increased in first 10 min of heating, then decreased thereafter ($p < 0.05$). Proportions of 15 and 9-*cis*-β-carotene changed within smaller ranges than proportions of TBC and 13-*cis*-β-carotene. Thus, 15 and 9-*cis*-β-carotene were probably less susceptible to thermal treatment than TBC and 13-*cis*-β-carotene.

The composition change of carotene with respect to heating time in purified oil is shown in Fig. 4B. The major isomers produced were 15-*cis*, 13-*cis*, and 9-*cis*-β-carotenes. Patterns of proportion change of carotene isomers in air and in purified oil were similar. Patterns of proportional change of carotene isomers in air and in purified oil were not significantly ($p > 0.05$) different. However, isomerization of carotene in purified oil occurred more rapidly than in air.

Thermal isomerization of carotenes in pumpkin Contents of individual pumpkin carotenes are shown in Fig. 5. The total carotene content in fresh pumpkin was 230 ± 6.1 µg/g of wet pumpkin, and was composed of TBC (120 ± 3.2 µg/g), TAC (70 ± 1.9 µg/g), and 9-*cis*-β-carotene (40 ± 1.1 µg/g). After blanching, the total carotene content decreased to 180 ± 5.2 µg/g of wet pumpkin, and to 190 ± 5.5 µg/g of wet pumpkin after retort treatment. Total carotene contents were not significantly ($p > 0.05$) affected by heating methods. The pumpkin matrix probably protected embedded carotenes at high

temperatures (13). Proportions of TBC and TAC in pumpkin decreased significantly ($p < 0.05$) as heating time increased. However, effects of different heating methods were not significantly ($p > 0.05$) different. The proportion of 13-*cis*-β-carotene increased after heat treatment, probably due to thermal isomerization of TBC to *cis*-isomers, and decreases in TBC and TAC contents (19).

In conclusion, the environmental conditions of carotene affected thermal isomerization patterns of β-carotenes. Thermal processing accelerated degradation of carotenes and affected isomerization of β-carotenes in pumpkin.

Disclosure The author declares no conflict of interest.

References

- Albanes D, Heinonen OP, Taylor PR, Virtamo J, Edwards BK, Rautalahti M, Hartman AM, Palmgren J, Freedman LS, Haapakoski J, Barrett MJ, Pietinen P, Malila N, Tala E, Liippo K, Salomaa E, Tangrea JA, Teppo L, Askin FB, Taskinen E, Erozan Y, Greenwald P, Huttunen JK. α-Tocopherol and β-carotene supplements and lung cancer incidence in the alpha-tocopherol, beta-carotene cancer prevention study: effects of base-line characteristics and study compliance. *J. Natl. Cancer I.* 88: 1560-1570 (1996)
- Mayne ST. Beta-carotene, carotenoids, and disease prevention in humans. *FASEB J.* 10: 690-701 (1996)
- Pietinen P, Ascherio A, Korhonen P, Hartman AM, Willett WC, Albanes D, Virtamo J. Intake of fatty acids and risk of coronary heart disease in a cohort of Finnish men: the alpha-tocopherol, beta-carotene cancer prevention study. *Am. J. Epidemiol.* 145: 876-887 (1997)
- Bendich A. Carotenoids and the immune response. *J. Nutr.* 119: 112-115 (1989)
- Mortensen A, Skibsted LH. Importance of carotenoid structure in radical-scavenging reactions. *J. Agr. Food Chem.* 45: 2970-2977 (1997)
- Lee J, Koo N, Min DB. Reactive oxygen species, aging, and antioxidative nutraceuticals. *Compr. Rev. Food Sci. F.* 3: 21-33 (2004)
- Ha DO, Park CU, Kim MJ, Lee J. Antioxidant and prooxidant activities of β-carotene in accelerated autoxidation and photosensitized model systems. *Food Sci. Biotechnol.* 21: 607-611 (2012)
- Stahl W, Sies H. Carotenoids and protection against solar UV radiation. *Skin Pharmacol. Appl.* 15: 291-296 (2002)
- Marx M, Stuparic M, Schieber A, Carle R. Effects of thermal processing on trans-*cis*-isomerization of β-carotene in carrot juices and carotene-containing preparations. *Food Chem.* 83: 609-617 (2003)
- Hiranvarachai B, Suvarnakuta P, Devahastin S. Isomerisation kinetics and antioxidant activities of β-carotene in carrots undergoing different drying techniques and conditions. *Food Chem.* 107: 1538-1546 (2008)
- Chen BH, Huang JH. Degradation and isomerization of chlorophyll *a* and β-carotene as affected by various heating and illumination treatments. *Food Chem.* 62: 299-307 (1998)
- Achir N, Pénicaud C, Avallone S, Bohuon P. Insight into β-carotene thermal degradation in oils with multiresponse modeling. *J. Am. Oil Chem. Soc.* 88: 2035-2045 (2011)
- Knockaert G, Puliserry SK, Lemmens L, Van Buggenhout S, Hendrickx M, Van Loey A. Carrot β-carotene degradation and isomerization kinetics during thermal processing in the presence of oil. *J. Agr. Food Chem.* 60: 10312-10319 (2012)
- Juna HI, Leeb CH, Songc GS, Kima YS. Characterization of the pectic polysaccharides from pumpkin peel. *LWT-Food Sci. Technol.* 39: 554-561 (2006)
- Murkovic M, Mülleder U, Neunteufl H. Carotenoid content in different varieties of pumpkins. *J. Food Compos. Anal.* 15: 633-638 (2002)
- Azizah AH, Wee KC, Azizah O, Azizah M. Effect of boiling and stir frying on total phenolics, carotenoids and radical scavenging activity of pumpkin (*Cucurbita moschata*). *Int. Food Res. J.* 16: 45-51 (2009)
- Canet W. Quality and stability of frozen vegetables. pp. 1-50. In: *Developments in food preservation vol. 5*. Thorne S. (ed). Elsevier, Philadelphia, PA, USA (1989)
- Obogh G. Effect of blanching on the antioxidant properties of some tropical green leafy vegetables. *LWT-Food Sci. Technol.* 38: 513-517 (2005)
- Gonçalves EM, Pinheiro J, Abreu M, Brandão TRS, Silva CL. Modelling the

- kinetics of peroxidase inactivation, colour and texture changes of pumpkin (*Cucurbita maxima* L.) during blanching. J. Food Eng. 81: 693-701 (2007)
20. Kumar MA, Ramesh MN, Rao SN. Retrofitting of a vertical retort for on-line control of the sterilization process. J. Food Eng. 47: 89-96 (2001)
 21. Simpson R, Almonacid S, Teixeira A. Optimization criteria for batch retort battery design and operation in food canning-plants. J. Food Process Eng. 25: 515-538 (2002)
 22. Gil B, Yoon SH. Thermooxidative degradation and isomerization of β -carotene during heating. Food Sci. Biotechnol. 8: 357-360 (1999)
 23. Jung MY, Min DB. Effects of quenching mechanisms of carotenoids on the photosensitized oxidation of soybean oil. J. Am. Oil Chem. Soc. 68: 653-658 (1991)
 24. Sadler G, Davis J, Dezman D. Rapid extraction of lycopene and β -carotene from reconstituted tomato paste and pink grapefruit homogenates. J. Food Sci. 55: 1460-1461 (1990)
 25. Zechmeister L, *cis-trans* Isomerization and stereochemistry of carotenoids and diphenylpolyenes. Chem. Rev. 34: 267-344 (1944)
 26. Quackenbush W. Reverse phase HPLC separation of *cis*- and *trans*-carotenoids and its application to *cis-trans* isomers of β -carotenes in food materials. J. Liq. Chromatogr. 10: 643-653 (1987)
 27. O'Neil CA, Schwartz SJ, Catignani GL. Comparison of liquid chromatographic methods for determination of *cis-trans* isomers of beta-carotene. J. Assoc. Off. Anal. Chem. 74: 36-42 (1991)
 28. de Carvalho LMJ, Gomes PB, Godoy RLO, Pacheco S, do Monte PHF, de Carvalho JLV, Nutti MR, Neves ACL, Vieira ACR, Ramos SRR. Total carotenoid content, α -carotene and β -carotene, of landrace pumpkins (*Cucurbita moschata* Duch): A preliminary study. Food Res. Int. 47: 337-340 (2012)
 29. Seo JS, Burri BJ, Quan Z, Neidlinger TR. Extraction and chromatography of carotenoids from pumpkin. J. Chromatogr. A. 1073: 371-375 (2005)
 30. Rodriguez EB, Rodriguez-Amaya DB. Formation of apocarotenals and epoxycarotenoids from β -carotene by chemical reactions and by autooxidation in model systems and processed foods. Food Chem. 101: 563-572 (2007)
 31. Mordt RC, Walton JC, Burton GW, Hughes L, Keith IU, David LA, Douglas MJ. Oxidative degradation of β -carotene and β -apo-8-carotenal. Tetrahedron 49: 911-928 (1993)
 32. Jung MY, Yoon SH, Min DB. Effects of processing steps on the contents of minor compounds and oxidation of soybean oil. J. Am. Oil Chem. Soc. 66: 118-120 (1989)
 33. Zeb A, Murkovic M. Determination of thermal oxidation and oxidation products of β -carotene in corn oil triacylglycerols. Food Res. Int. 50: 534-544 (2013)
 34. Onyewu PN, Daun H, Ho CT. Formation of two thermal degradation products of beta-carotene. J. Agr. Food Chem. 30: 1147-1151 (1982)
 35. Yoon SH, Jung MY, Min DB. Effects of thermally oxidized triglycerides on the oxidative stability of soybean oil. J. Am. Oil Chem. Soc. 65: 1652-1656 (1988)
 36. Henry LK, Catignani G, Schwartz S. Oxidative degradation kinetics of lycopene, lutein, and 9-*cis* and all-*trans*- β -carotene. J. Am. Oil Chem. Soc. 75: 823-829 (1998)