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The contents of β-carotene and cholesterol in selected types of agricultural and processed foods in Korea

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Abstract

In this study, the contents of β-carotene and cholesterol were evaluated in various types of agricultural and processed foods (vegetables, legume products, dairy products, and eggs). Certified reference material (CRM) with an assigned value was used for the validation of the β-carotene and cholesterol analytical methods. High recoveries (accuracy) of β-carotene (96%) and cholesterol (97%) were obtained from the quantitative analysis of the CRM, with a relative standard deviation (%) of 1.86 and 3.35% for the β-carotene and cholesterol, respectively. Vegetables contained relatively high concentrations of β-carotene (raw *Toona sinensis*, 1650.97 μg/100 g, a raw small onion, 879.09 μg/100 g, and a raw lettuce stem, 591.89 μg/100 g). The β-carotene values in dried chickpeas (22.94 μg/100 g) and dried brown lentils (21.98 μg/100 g) were similar. The highest β-carotene value among the analyzed dairy products was found in banana milk (234.21 μg/100 g) while other flavored products (strawberry milk and chocolate milk) did not contain any β-carotene. Furthermore, β-carotene was not detected in goat milk and high calcium milk in this study. With regard to cholesterol among the analyzed samples, the highest cholesterol value was found in egg yolk (629.30 mg/100 g), and cooking methods (boiling and frying) had little effect on the cholesterol levels of eggs. In addition, the cholesterol content in vanilla ice cream was 28.77 mg/100 g which was the highest value among the analyzed dairy products.

Keywords: agricultural foods, β-carotene, cholesterol, high performance liquid chromatography, processed foods

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Introduction

The major chronic diseases prevalent in human beings (hyperlipidemia, cancer, diabetes, cardiovascular disease, stroke, and coronary heart disease) are closely related to the daily diet. Consumption of fruits and vegetables that are rich in carotenoids and other bioactive components has been highly recommended to prevent the occurrence of these chronic diseases (Rao and Rao, 2007).

β-Carotene is the main ingredient of carotenoids in foods and it is widely used in nutritional supplements as a pro-vitamin A and antioxidant. In the previous study (Lynch et al., 2011), it was

proven that β-carotene can prevent mitochondrial DNA from being damaged. In terms of food pigment, β-carotene is abundant in yellow-orange vegetables and fruits (Mangels et al., 1993). Therefore, different types of carotenoids are responsible for lipid-soluble food colorants, and vegetables and fruits are the main dietary source of β-carotene in the human diet.

Cardiovascular diseases are closely related to plasma cholesterol concentration. However, cholesterol plays an important role in fat metabolism and physiological reactions in the body. In addition, it acts as a precursor of steroid hormones and vitamin D (Brown and London, 2000). Although cholesterol is mainly derived from biosynthesis in the body, the cholesterol content in dietary foods seems to be a factor that affects the serum cholesterol level. This phenomenon emphasizes that the determination of cholesterol content in foods of animal origin, such as dairy products and eggs, is important. Therefore, in order to facilitate a healthy diet by consuming high β-carotene and low cholesterol, determination of the content of the two components in agricultural foods is imperative.

The aim of this study was to determine the values of β-carotene and cholesterol in selected types of agricultural and processed foods (i.e., vegetables, legume products, dairy products, and eggs) in South Korea.

Materials and Methods

Materials and reagents

Selected types of agricultural and processed foods were provided from Rural Development Administration (Jeonju, Korea) which included vegetables (7 types), legume products (6 types), dairy products (7 types), and eggs (5 types). A food blender was used to homogenize each type of samples and further dividing them into two batches (n = 2) for β-carotene and cholesterol analysis. β-Carotene and cholesterol standards were purchased from Sigma-Aldrich (St. Louis, USA). 5α-Cholestane purchased from Sigma-Aldrich (St. Louis, USA) was used as an internal standard for cholesterol quantification. All solvents used in this study are HPLC grade unless otherwise specified.

Analysis of certified reference materials

Certified reference materials (CRM) of mixed vegetables and infant nutritional formula with assigned values were used for the validation of β-carotene and cholesterol analytical methods in this study, respectively. The recovery rate was calculated by comparing with known assigned values of β-carotene and cholesterol.

Extraction of β-carotene and cholesterol by alkali saponification from foods

Extraction of β-carotene and cholesterol from agricultural and processed foods was performed according to the National Laboratory System (NLS) method with slight modifications (Shin et al., 2016b; KFDA, 2017a). Each homogenized sample (3 g) was weighed and put into a 200-mL extraction tube, and 10 mL of 6% pyrogallol solution (in ethanol) was added. The mixture was then vortexed for 2 min, flushed with N_2 for 1 min, and sonicated for 10 min. Afterwards, 8 mL of 60% potassium hydroxide (KOH) solution was added, after vortexed for 2 min and flushed with nitrogen for 1 min, the samples were put in a shaking water bath at 75°C for 1 h at 100 rpm. After lowering the temperature of the extraction tube at cold water, 10 mL of 2% sodium chloride solution (in distilled water) was used to wash twice. Fifteen milliliters of extraction

solvent (n-hexane : ethyl acetate, 85 : 15, v/v, Butylated hydroxytoluene [BHT] 0.01%) were used to extract β-carotene and cholesterol three times. All supernatants were collected and pooled into a new volumetric flask (50 mL) through the anhydrous sodium sulfate column to remove moistures. The volumetric flask was filled up with extraction solvent and the final extraction solution was gently poured into a 50-mL vial with cap. And then it was preserved at - 20℃ until further analysis.

Determination of β-carotene content

β-Carotene content was measured by high performance liquid chromatography (HPLC) following NLS procedure with slight modifications (KFDA, 2017a). Ten milliliters of extraction solution were taken and flushed under nitrogen. Then, 1 mL of chloroform was added in order to dissolve the residues.

β-Carotene analysis by HPLC was carried out according to the previous study (Shin et al., 2015; Shin et al., 2016a). Twenty microliters of the analytical solution were injected into an HPLC (Younglin SP930D dual pump, Younglin, Anayang, Korea) equipped with a Shiseido Capcell Pak UG120 C18 column (5 μm, 250 × 4.6 mm i.d., Shiseido, Tokyo, Japan). Solvent A (acetonitrile : methanol : methylene chloride, 70 : 10 : 30, v/v/v) and solvent B (acetonitrile : methanol : methylene chloride, 75 : 20 : 5, v/v/v) were used as mobile phase. The total analysis time was 40 min. External calibration curve was used to determine β-carotene, which was prepared at concentrations of 0.1, 0.5, 1, 5, 10, 20, 40 μg/mL of β-carotene standard. The equation of the external calibration curve was $Y = 241.86X - 5.6768$, $R^2 = 0.9998$ (Y = peak area of β-carotene, X = concentration (μg/mL) of β-carotene). The results were shown as μg/100 g of agricultural and processed foods. The values were obtained from duplicate analysis of each food, and their averages, standard deviations (SD), and relative standard deviations (RSD) were calculated.

Determination of cholesterol content

Quantification of cholesterol was conducted with gas chromatography (GC) following NLS procedure with slight modifications (KFDA, 2017b). The extraction solution of 12.5 mL was taken out from the extraction solution of 50 mL and flushed with nitrogen. Three milliliters of acetone were added to further remove moisture and then flushed with nitrogen. After that, 3 mL of dimethyl formamide was added into the vials to dissolve the residues. After completely mixed, 1 mL solution was taken and derivatized with derivatization solution (0.2 mL of hexamethyldisilazane and 0.1 mL of chlorotrimethylsilane) at room temperature for 15 min. Finally, 1 mL of internal standard solution (IS: 5α-cholestane, 0.1 mg/mL in n-heptane) and 10 mL of distilled water were added to the test tube to terminate the derivatization. The mixture was thoroughly vortexed for 1 min and the supernatant was collected through an anhydrous sodium sulfate column for GC analysis.

Two microliters of samples were injected into a GC system (Younglin 6100, Younglin, Anyang, Korea) combined with a flame ionized detector (FID). Cholesterol was separated by a HP ultra-2 column (25 m × 0.25 mm, 0.33 μm), nitrogen was used as a carrier gas at a pressure of 18.4 psi, and the flow rate was set at 0.4 mL/min. The detector and injector temperatures were set at 300 and 280℃, respectively. The oven temperature was programmed initially at 260℃ for 2 min, raised to 285℃ at 10℃/min, then finally held for 10 min. An external calibration curve was used to quantitatively analyze the cholesterol content of samples, which were made by 0.0025, 0.005, 0.01, 0.05, 0.1, 0.2 mg/mL of cholesterol standard. The equation of the external calibration curve was $Y = 9.9337X + 0.0136$, $R^2 = 0.9994$ (Y = peak area ratio of cholesterol and internal standard,

X = concentration (mg/mL) of cholesterol). The results were shown as mg/100 g of agricultural and processed foods. Analysis of each food was carried out in duplicates, and their averages, standard deviations (SD), and relative standard deviations (RSD) were calculated.

Results and Discussion

Recovery rate of certified reference materials

The results of analyzing the β-carotene content of mixed vegetables (CRM 485) and the cholesterol content of infant nutritional formula (CRM 1849a) are shown in Table 1. In our study, the β-carotene content and cholesterol content in CRM 485 and CRM 1849a were 2370 μg/100 g and 13.35 mg/100 g, respectively. Compared to the certified concentration values of CRM 485 (2275 μg/100 g) and CRM 1849a (13.74 mg/100 g), high recovery rates (accuracy) of β-carotene (96%) and cholesterol (97%) were obtained from the quantitative analysis of CRMs (Table 1), indicating that the values given in this study were well in line with the recovery rates of β-carotene and cholesterol, which were from 90% to 110% (KFDA, 2017c). Furthermore, the RSD of β-carotene and cholesterol was 1.86% and 3.35%, respectively (Table 1).

β-Carotene content in selected types of vegetables and legume products

β-Carotene values in different types of vegetables are shown in Table 2. The highest content was found in raw *Toona sinensis* (1650.97 μg/100 g). In addition, β-carotene was not detected in the root of raw lettuce, but was detected in the stem of raw lettuce, indicating that β-carotene in lettuce was present only in the stem but not in the root (Table 2). Interestingly, the

Table 1. Recovery rates of β-carotene of certified reference material 485 (mixed vegetables) and cholesterol of certified reference material 1849a (infant nutritional formula).

Certified reference materials	β-Carotene (μg/100 g)	Cholesterol (mg/100 g)
	Mixed vegetables	Infant nutritional formula
Certified concentration value	2,275 ± 420	13.74 ± 0.29
Analysis value	2,370 ± 150	13.35 ± 0.45
RSD (%)	1.86	3.35
Recovery (%)	96	97

RSD, relative standard deviation.

Table 2. β-Carotene content of selected vegetables.

Description	β-Carotene (μg/100 g)		
	Mean	SD	RSD
Lettuce, root, raw	ND	-	-
Lettuce, stem, raw	591.89	1.44	0.24
Bean sprouts, raw	5.76	1.50	25.96
Bean sprouts, boiled	35.12	1.22	3.48
Onion, welsh, raw	277.47	29.01	10.46
Onion, small, raw	879.09	57.67	6.56
Toona sinensis, raw	1650.97	138.87	8.41

SD, standard deviation; RSD, relative standard deviation (%); ND, not detected.

β-carotene content in raw small onion (879.09 μg/100 g) was 3.17 times higher than that in raw Welsh onion (277.47 μg/100 g), suggesting that raw small onion is a better diet source than raw Welsh onion. On the other hand, the β-carotene value of boiled bean sprouts (35.12 μg/100 g) was higher than that in raw bean sprouts (5.76 μg/100 g). In general, β-carotene was embedded in a matrix with protein in vegetables. During the boiling process, the peptide chain structures of proteins may be denatured (Lee et al., 2001). Along with this phenomenon, tissues loosened due to the boiling process may accelerate β-carotene recovery rates during extraction.

The β-carotene content in selected legume products is shown in Table 3. The β-carotene value found in dried soybean was 5.66 μg/100 g, but β-carotene was not detected in soybean soup and black soymilk. It seems that the production process can be responsible for such differences, in which β-carotene may be destroyed due to high temperature because it is sensitive to light and heat. In addition, the β-carotene value of dried brown lentils (21.98 μg/100 g) was found to be similar to dried chickpeas (22.94 μg/100 g) but was higher than that in dried red lentils (11.30 μg/100 g).

β-Carotene and cholesterol contents of selected dairy products and eggs

The contents of β-carotene and cholesterol were determined in different types of dairy products (Table 4). The highest β-carotene value among the analyzed dairy products was found in banana milk (234.21 μg/100 g), while other flavored products (strawberry milk and chocolate milk) did not contain β-carotene. These differences may be due to the addition of β-carotene as a food colorant in banana milk. Also, β-carotene was not detected in goat milk and high calcium milk in this

Table 3. β-Carotene content of selected legume products.

Description	β-Carotene (μg/100 g)		
	Mean	SD	RSD
Soybean, dried	5.66	0.38	6.67
Soybean, soup	ND	-	-
Soymilk, black soybean	ND	-	-
Lentils, red, dried	11.30	0.24	2.16
Lentils, brown, dried	21.98	1.82	8.29
Chickpeas, dried	22.94	3.03	13.20

SD, standard deviation; RSD, relative standard deviation (%); ND, not detected.

Table 4. The contents of β-carotene and cholesterol in selected dairy products.

Description	β-Carotene (μg/100 g)			Cholesterol (mg/100 g)		
	Mean	SD	RSD	Mean	SD	RSD
Milk						
Coffee	2.90	0.58	20.06	6.78	0.16	2.39
Strawberry	ND	-	-	5.47	0.04	0.77
Chocolate	ND	-	-	5.51	0.02	0.35
Banana	234.21	6.26	2.67	11.91	0.03	0.26
Goat	ND	-	-	16.47	0.05	0.31
High calcium	ND	-	-	14.61	0.32	2.18
Ice cream						
vanilla	4.85	0.14	2.83	28.77	1.67	5.80

SD, standard deviation; RSD, relative standard deviation (%); ND, not detected.

Table 5. The contents of β-carotene and cholesterol in eggs.

Description	β-Carotene (μg/100 g)			Cholesterol (mg/100 g)		
	Mean	SD	RSD	Mean	SD	RSD
Egg						
raw	ND	-	-	328.83	3.42	1.04
boiled	4.50	0.83	18.34	305.56	15.20	4.98
fried	ND	-	-	357.34	1.06	0.30
white, raw	ND	-	-	ND	-	-
yolk	12.14	1.50	12.35	629.30	30.06	4.78

SD, standard deviation; RSD, relative standard deviation (%); ND, not detected.

study (Table 4). In coffee milk, a very small amount of β-carotene (2.90 μg/100 g) was determined. Furthermore, the β-carotene value of vanilla ice cream (4.85 μg/100 g) was slightly lower than that in the USDA National Nutrient Database (USDA, 2018), in which the β-carotene value of vanilla ice cream was 19 μg/100 g. The difference could be due to the different sources of original milk, production process, etc.

With regard to cholesterol, the cholesterol content (16.47 mg/100 g) in goat milk was slightly higher than that in the others (coffee milk, strawberry milk, chocolate milk, banana milk, and high calcium milk). It is known that the nutritional composition of milk is affected by many factors such as season, stages of lactation, diet and individual animal (Park and Chukwu, 1988; Park, 1990). In addition, the cholesterol content in vanilla ice cream was 28.77 mg/100 g, which was the highest value among the analyzed dairy products.

According to the USDA National Nutrient Database (USDA, 2018), β-carotene was not detected in raw whole egg and raw egg white, which is well in agreement with our results. While egg yolk showed 88 μg/100 g, a relatively low amount was observed in our study, in which 12.14 μg/100 g of β-carotene was detected in egg yolk (Table 5). Furthermore, a high cholesterol content in egg yolk (629.30 mg/100 g) was observed, while egg white did not contain cholesterol. Therefore, a raw egg had 328.83 mg/100 g of cholesterol, as expected. Also, it seems that the boiling and frying processes did not much affect the content of cholesterol in egg, in which 305.56 - 357.34 mg/100 g was observed.

It is known that β-carotene exists as a form of carotenoid-protein complexes in most foods. When heated, the matrix may be broken down, and β-carotene is released. Therefore, increases in extraction rates can be expected. A very small amount of β-carotene was observed in boiled egg (4.50 μg/100 g, and even this value is a very small amount), while it was not found in raw egg or fried egg. The differences were considered to be due to different cooking parameters (temperature, cooking time, etc.). In the case of cholesterol, however, the cholesterol content in raw egg was not very different from boiled and fried eggs, suggesting that different cooking methods (boiling and frying) have little effect on the cholesterol content of cooked eggs.

Conclusion

β-Carotene and cholesterol contents in seven types of vegetables, six types of legume products, seven types of dairy products and five types of eggs were determined in this study. The highest β-carotene value was found in raw *Toona sinensis* (1650.97 μg/100 g), followed by raw small onion (879.09 μg/100 g) and raw lettuce stem (591.89 μg/100 g). Legume products contain a relatively low β-carotene level (chickpeas, dried, 22.94 μg/100 g; lentils, brown, dried, 21.98 μg/100 g; lentils, red, dried, 11.30 μg/100 g; soybean, dried, 5.66 μg/100 g). In the case of cholesterol, the highest concentration was found in egg

yolk, and no significantly different cholesterol content was found in eggs depending on the cooking method (boiling or frying).

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