Food Sci Biotechnol (2018) 27(2):333–342
https://doi.org/10.1007/s10068-017-0281-1

Effect of different cooking methods on the content of vitamins and true retention in selected vegetables

Seongeung Lee[1] · Youngmin Choi[2] · Heon Sang Jeong[1] · Junsoo Lee[1] · Jeehye Sung[1]

Received: 25 May 2017 / Revised: 17 November 2017 / Accepted: 2 December 2017 / Published online: 12 December 2017

Abstract This study evaluated the effect of different cooking methods including blanching, boiling, microwaving and steaming on the content of vitamins in vegetables. True retention was estimated using the yield expressed as a ratio of the weight of the cooked sample to the weight of the raw sample. The retention of vitamin C ranged from 0.0 to 91.1% for all cooked samples. Generally, higher retention of vitamin C was observed after microwaving with the lowest retention recorded after boiling. Cooked vegetables were occasionally higher contents of fat-soluble vitamins, including α-tocopherol and β-carotene, than that of their fresh counterparts, but it depends on the type of vegetables. Microwave cooking caused the greatest loss of vitamin K in crown daisy and mallow; in contrast, it caused the least loss of vitamin K in spinach and chard. Cooking may cause changes to the contents of vitamins, but it depends on vegetables and cooking processes.

Keywords Vegetables · Cooking · Retention · Vitamins

✉ Jeehye Sung
jeehye@chungbuk.ac.kr

[1] Division of Food and Animal Sciences, College of Agriculture, Life, and Environmental Sciences, Chungbuk National University, 1 Chungdae-ro, Seowon-gu, Cheongju, Chungbuk 28644, Korea

[2] National Academy of Agricultural Science, Rural Development Administration, Jeonju, Jeonbuk 55365, Korea

Introduction

Vitamins are organic compounds and vital nutrients that cannot be synthesized and thus must be obtained through the diet. Although vitamins are usually needed in minute amounts for normal physiological functions such as maintenance, growth, and development, insufficient intake of vitamins gives rise to specific deficiency syndromes [1]. Many reports have also found various health benefits of vitamins. In detail, ascorbic acid and α-tocopherol have anti-aging and redox state regulation effects as powerful antioxidants [2, 3], α-tocopherol and β-carotene could be synergistic antioxidants and may prevent several cancers [4–6], and vitamin K intake is associated with reduced cardiovascular disease and vascular calcification [7].

Vegetables are excellent sources of vitamins including β-carotene (provitamin A), ascorbic acid, and vitamins E and K [8]. Several epidemiological studies have suggested that diets rich in vegetables are associated with reduced risks of chronic diseases [9]. Therefore, consumption of vegetables has rapidly increased in recent years due to their health benefits [10]. Prior to consumption, vegetables are usually cooked using heat treatments such as steaming, blanching, boiling, and microwaving. It is well known that cooking alters the nutritional value of fresh vegetables. However, whilst several studies have focused on how cooking changes the nutritional components and phytochemical content of vegetables, few have investigated the true retention of vitamins following exposure to different cooking methods [11–13]. Additionally, cooking or heat treatments can have a significant impact on the content of vitamins, and lead to an inaccurate estimation of nutrient intake. Therefore, it is necessary to establish nutritional information on the retention of vitamins from vegetables by the different processing methods. The true retention

is an important component defining the ultimate importance of vitamins in the consumed vegetables.

The objectives of this study were to investigate the effect of different cooking methods including blanching, boiling, microwaving, and steaming, on the content and true retention of vitamins (i.e. β-carotene, ascorbic acid and vitamins E and K) in vegetables. In addition, analytical method validation parameters such as accuracy and precision were determined to ensure the validity of vitamin analysis.

Materials and methods

Materials

Ten vegetables including broccoli, chard, mallow, potato, sweet potato, carrot, crown daisy, pellia leaf, spinach, and zucchini were purchased from a local retail market (Chungbuk, Korea). Vitamin K and β-carotene were obtained from Waco Pure Chemical Industries (Osaka, Japan). Sodium acetate, acetic acid, zinc powder (particle size < 60 μm), α-tocopherol and γ-tocopherol were purchased from Merck (Darmstadt, Germany). Ascorbic acid, monobasic potassium phosphate, metaphosphoric acid, potassium hydroxide, and potassium carbonate were purchased from Sigma-Aldrich (St. Louis, MO, USA). All reagents and solvents were analytical and high performance liquid chromatography (HPLC) grade.

Cooking methods

The fresh vegetables were cleaned, washed, and cut into pieces before cooking treatment. All vegetables have been well done but firm to the bite. After removing the main part of the stem, the broccoli was cut into floweret pieces. The width, height, and length of the pieces were 4, 4, and 8, respectively. The carrot, potato and sweet potato were divided into quarters and cut into many cubes 2 cm on a side. The zucchini cut into rectangular pieces which has a width of 4 cm, a height of 2 cm, and a length of 4 cm. The detailed cooking conditions were listed in Table 1. Four different cooking methods were tested: boiling, blanching, steaming, and microwaving. For boiling, vegetables were added to distilled water that had just reached the boil in a glass pot (1:5, food/water). Cooking time was 5 min for broccoli, chard, mallow, carrots, sweet potato, crown daisy, perilla leaf, and spinach, and 20 min for potato and zucchini. For blanching, vegetables were added to boiled distilled water in a glass pot (1:5, food/water) and cooked on a moderate flame. Cooking time was 1 min for broccoli, chard, mallow, zucchini, crown daisy, sweet potato, perilla leaf, and spinach, 3 min for carrots, and 5 min for potato. After boiling and blanching treatments, samples were drained, stored at − 80 °C and lyophilized. For steaming, vegetables were steamed in a closed stainless steel pot using a stainless-steel steam basket above boiling distilled water for 5 ∼ 20 min. Microwaving was carried out in a domestic microwave oven (Samsung, Suwon, Republic of Korea) without water. Sample were placed in a glass dish on the rotating plate of the oven and exposed to full power (700 W; 2452 MHz) for 2–5 min. After steaming and microwaving treatments, samples were stored at − 80 °C and lyophilized.

Analysis of vitamins C

Lyophilized samples of raw and cooked vegetables (0.2 g) were ground and added to 30 mL of 3% metaphosphoric acid solution and homogenized at 11,000 rpm for 2 min using a T25 basic ULTRA-TURRAX® homogenizer (IKA-Werke GmbH & Co. KG, Staufen, Germany). The volume was made up to 50 mL with 3% metaphosphoric acid solution. The extract (2 mL) was centrifuged at 12,000 rpm for 3 min, and the supernatant filtered through a 0.45 μm polyvinylidene difluoride (PVDF) membrane filter (Whatman International Ltd., Maidstone, UK). All samples were immediately analyzed using an HPLC system, equipped with a PU-2089 pump, an AS-2057 auto injector, and a MD-2010 UV–vis variable wavelength detector (JASCO Corp., Tokyo, Japan). Separation was carried out in a CrestPak C18S column (150 × 4.6 mm, i.d., 5 μm, JASCO Corp.), and the isocratic elution was carried out with 0.1% trifluoroacetic acid in distilled water as a mobile phase for 15 min (flow rate 0.8 mL/min). The peak was read at 254 nm using an UV detector and quantification was determined via external calibration against ascorbic acid.

Analysis of vitamins E

Vitamin E content was determined using saponification extraction as outlined by Lee et al. [14], but with some modifications. Lyophilized samples (1.0 g) were ground and mixed with 20 mL of ethanol containing pyrogallol (6% wt/vol) and 8 mL of potassium hydroxide solution (60% wt/vol) and heated at reflux (70 °C, 50 min) with shaking in a water bath (NTS-1300, Eyela, Japan). The mixture was cooled in an ice bath and 20 mL of 2% sodium chloride solution and 20 mL of *n*-hexane:ethyl acetate (85:15, v/v) containing 0.1% butylated hydroxytoluene (BHT) were added as the extracting solvent. After vortexing for 2 min, the phases were allowed to separate, and extracted three times with 20 mL portions of the extracting solvent. The organic phases were collected and made up to 50 mL and the resulting solution, containing vitamin E,

Table 1 Cooking condition of vegetables

Common name	Scientific name	Boiling time (min)[a]	Blanching time (min)[b]	Steaming time (min)[c]	Microwaving time (min)[d]
Broccoli	*Brassica oleracea*	5	1	10	2
Chard	*Beta vulgaris*	5	1	10	3
Mallow	*Amaranthus tricolor*	5	1	10	2
Potato	*Solanum tuberosum*	20	5	20	3
Sweet potato	*Ipomoea batatas*	20	5	20	3
Carrot	*Daucus carota*	12	3	15	4
Crown daisy	*Chrysanthemum coronarium*	5	1	10	1
Perilla leaf	*Perilla frutescens*	5	1	10	1
Spinach	*Spinacia oleracea*	5	1	10	3
Zucchini	*Cucurbita pepo*	12	3	15	4

[a]Boiled with 500 mL boiling distilled water and put it in the colander to drain for 2 min

[b]Blanched with 500 mL boiling distilled water and put it in the colander to drain for 2 min

[c]After adding distilled water in a steamer and boiled, steamed over medium heat

[d]Placed in microwave and cooked on both side

was evaporated under nitrogen gas. The residues were redissolved in *n*-hexane, filtered through a 0.45 μm polytetrafluoroethylene (PTFE) membrane filter (Whatman) and analyzed using an HPLC system equipped with a PU-2089 pump, an AS-2057 auto injector, and an FP-2020 fluorescence detector (JASCO Corp.). Analysis of vitamin E was performed on a LiChrosphere® Diol 100 column (250 × 4 mm, i.d., 5 μm, Merck, Berlin, Germany) using a mobile phase of hexane/isopropanol (98.7:1.3, v/v) at a flow rate of 1.0 mlL/min. Peaks were detected by fluorescence using an excitation wavelength of 290 nm and an emission wavelength of 330 nm. Quantification was determined via an external calibration against α- and γ-tocopherols.

Analysis of vitamins K

Vitamin K was determined using the solvent extraction method outlined by Jakob and Elmadfa [15], but with some modifications. Lyophilized samples of raw and cooked vegetables (1 g) were ground and added to 30 mL of dichloromethane: methanol (2:1, v/v) and homogenized at 11,000 rpm for 2 min using a T25 basic ULTRA-TURRAX® homogenizer. The extract was made up to 50 mL with methanol and 2 mL aliquot of the extract was transferred to glass tubes and evaporated under nitrogen gas. The residue was redissolved with *n*-hexane, and 8 mL of methanol:water (9:1, v/v) was added prior to centrifuging for 5 min at 2,000 rpm. The hexane layer was collected and evaporated to dryness. The residue was redissolved with methanol, filtered through a 0.45 μm PTFE membrane, and analyzed using an HPLC system equipped with a PU-2089 pump, an AS-2057 auto injector, and an FP-2020 fluorescence detector (JASCO Corp.). Analysis of vitamin K was performed on a Zorbax Eclipse XDB-C18 column (150 × 4.6 mm, i.d., 5 μm, Agilent, Palo Alto, USA) using a mobile phase of methanol:dichloromethane (9:1, v/v) and a flow rate of 1.0 mL/min. Peaks were detected by fluorescence using an excitation wavelength of 243 nm and an emission wavelength of 430 nm. Quantification was determined via an external calibration against phylloquinone.

Analysis of β-carotene

Lyophilized samples (1.0 g) were ground and carried out saponification for extraction of β-carotene from vegetables, as previously described by Lee et al. [14], but with some modifications. All samples were analyzed using an HPLC system equipped with a PU-2089 pump, an AS-2057 auto injector, and an MD-2010 UV–Vis variable wavelength detector (JASCO Corp.). Separation was carried out in a CrestPak C18S column (150 × 4.6 mm, i.d., 5 μm, JASCO Corp.). Analysis of β-carotene was performed on an Ascentis RP-Amide column (150 × 4.6 mm, i.d., 5 μm, Supelco, Bellefonte, USA) using a mobile phase of acetonitrile:methanol (7:3, v/v) and a flow rate of 1.0 mL/min. Peaks were measured at 473 nm using a UV detector and quantification was determined by external calibration against β-carotene.

Determination of true retention during cooking

True retention (TR) values for all vitamins were calculated using the following formula [16]:

$$\text{TR}\ (\%) = \frac{\text{Nutrient content per g of cooked food} \times \text{g of food after cooking}}{\text{Nutrient content per g of raw food} \times \text{g of food before cooking}} \times 100$$

Method validation

All analysis methods were validated by determining the precision (repeatability and reproducibility) and accuracy (recovery) [14]. The precision of the assay was defined as the coefficient of variation (CV); there were at least five repetitions. The recovery was calculated by the following equation: $R\% = [(Cs - Cp)/Ca] \times 100$, where R (%) is the recovery of added standard; Cs the vitamin content in spiked sample; Cp the vitamin content in sample; Ca the vitamin added.

Statistical analysis

One-way analysis of variance (ANOVA) was performed using SAS version 9.4 (SAS Institute, Cary, USA). Results were compared using Duncan's test with a significance level of $p < 0.05$.

Results and discussion

The effect of cooking methods on vitamin C content in vegetables

The nutritional importance of vitamin C (L-ascorbic acid; 2,3-endiol-L-gulonic acid-γ-lactone) as an essential water-soluble vitamin is well established. Vitamin C is a cofactor in numerous physiological reactions, including collagen gene expression, peptide hormone activation, and carnitine synthesis; it is also an effective antioxidant. Therefore, adequate intake of vitamin C from food is vital for normal functioning of the human body [17]. In this study, Table 2 shows the content of vitamin C in raw and cooked vegetables after exposure to different cooking methods. Vitamin C content varied widely between the raw vegetables tested; it was not detected in mallow, crown daisy, and perilla leaf with the lowest content found in carrots (39.92 mg/kg of fresh weight), and the highest in broccoli (668.04 mg/kg of fresh weight). These findings are consistent with the results of previous investigations by Bureau et al. [18], reporting that raw broccoli was a higher in vitamin C content than most other raw vegetables such as spinach and carrot, with levels of 583, 237, 107, and 19 mg/kg of fresh weight reported for broccoli, spinach, zucchini, and carrot, respectively. Concentration of vitamin C in raw vegetables in the present study were higher than those reported by Bureau et al. [18], but these differences might be due to the fact that Bureau et al. [18] used frozen vegetables. Also, it is well known that vitamin content is varied with cultivar and growing conditions. Vitamin C is a water-soluble and temperature-sensitive vitamin, so is easily degraded during cooking, and elevated temperatures and long cooking times have been found to cause particularly severe losses of vitamin C [12]. In this study, boiling destroyed vitamin C in almost all the samples, with nutrient retention ranging from 0 to 73.86%; the greatest loss was found in boiled chard. Blanching also destroyed vitamin C in the samples, indicated by the retention that ranged from 57.85 to 88.86%, with the greatest loss found in blanched spinach. Steaming treatment significantly reduced the retention of vitamin C in all vegetables except broccoli; retention ranged from 0 to 89.24%. Microwaving had less of an impact on vitamin C content, with high retention (> 90%) observed for spinach, carrots, sweet potato, and broccoli. Steaming and microwaving retained higher concentrations of vitamin C than boiling because of the reduced contact with water at relatively low temperatures. Using minimal cooking water and cooking for shorter time periods should result in higher vitamin C retention.

The effect of cooking methods on vitamin K content in vegetables

Vitamin K, a fat-soluble vitamin, is well known for its beneficial role in blood coagulation and bone metabolism. Although phylloquinone (vitamin K1) and menaquinone (vitamin K2) are the two naturally occurring forms of vitamin K, phylloquinone levels in vegetables and fruits range from extremely low to quite high [19]. In this study, the content and true retention of vitamin K in raw vegetables and their changes after cooking are shown in Table 2. The highest amounts of vitamin K were found in green leafy vegetables (chard, mallow, crown daisy, perilla leaf and spinach; 1.59, 3.41, 1.19, 3.18, and 2.34 mg/kg of fresh weight, respectively) and green flowers (broccoli; 1.54 mg/kg of fresh weight). However, root vegetables including potato and sweet potato had low amounts of vitamin K. This is expected, as it is well known that phylloquinone is found at highest concentrations in green leafy vegetables [20]. The retention of vitamin K in cooked vegetables ranged from 44.28 to 216.65%, and our results show that microwaving caused the highest loss of vitamin K in crown daisy and mallow but the lowest loss in spinach and chard. Cooking fresh chard and perilla leaf lead to a significant change in vitamin K content, with a trend towards higher concentrations of vitamin K in cooked vegetables than in the corresponding raw samples, although this was not consistent across all comparisons. Similar results were described by Damon et al. [21] for several

Table 2 The contents and true retention of vitamin C and vitamin K in fresh and cooked vegetables

Sample	Cooking treatment	Vitamin C		Vitamin K	
		CW[a] (mg/kg)	TR[b] (%)	CW[a] (mg/kg)	TR[b] (%)
Broccoli	Raw	668.04[c]	100.00[b]	1.54[a]	100.00[a]
	Boiled	370.04[d]	52.85[d]	1.59[a]	98.89[a]
	Blanched	615.42[c]	88.86[c]	1.69[a]	108.81[a]
	Steamed	761.48[b]	111.21[a]	1.90[a]	123.08[a]
	Microwaved	836.15[a]	112.76[a]	1.71[a]	101.76[a]
Chard	Raw	163.04[a]	100.00[a]	1.59[c]	100.00[b]
	Boiled	0.00[c]	0.00[c]	2.95[a]	116.23[a]
	Blanched	124.58[b]	59.69[b]	2.38[b]	116.73[a]
	Steamed	0.00[c]	0.00[c]	2.24[b]	105.60[ab]
	Microwaved	173.43[a]	67.64[b]	2.82[a]	112.62[ab]
Mallow	Raw	ND[c]	ND	3.41[a]	100.00[ab]
	Boiled	ND	ND	3.00[a]	81.75[b]
	Blanched	ND	ND	2.17[b]	56.71 [cd]
	Steamed	ND	ND	2.66[ab]	69.33[bc]
	Microwaved	ND	ND	2.03[b]	44.28[d]
Potato	Raw	58.30[a]	100.00[a]	0.02[b]	100.00[b]
	Boiled	30.57[c]	49.79[c]	0.03[a]	141.16[a]
	Blanched	44.73[b]	73.20[b]	0.02[b]	94.71[b]
	Steamed	51.08[b]	83.65[b]	0.03[a]	75.98[c]
	Microwaved	62.98[a]	76.86[b]	0.03[ab]	95.32[b]
Sweet potato	Raw	131.35[b]	100.00[a]	ND	ND
	Boiled	95.20[c]	73.86[b]	ND	ND
	Blanched	88.92[c]	67.70[bc]	ND	ND
	Steamed	81.32[c]	59.44[c]	ND	ND
	Microwaved	183.73[a]	100.21[a]	ND	ND
Carrot	Raw	39.92[b]	100.00[a]	0.04[b]	100.00[a]
	Boiled	56.62[d]	55.33[c]	0.04[b]	85.02[ab]
	Blanched	32.15 [cd]	72.71[b]	0.03[b]	71.86[b]
	Steamed	33.49[bc]	70.51[b]	0.04[b]	69.87[b]
	Microwaved	63.00[a]	92.02[a]	0.06[a]	85.35[ab]
Crown daisy	Raw	ND	ND	1.19[c]	100.00[a]
	Boiled	ND	ND	1.70[a]	100.11[a]
	Blanched	ND	ND	1.31[bc]	89.67[a]
	Steamed	ND	ND	1.54[ab]	89.42[a]
	Microwaved	ND	ND	0.73[d]	49.80[b]
Perilla leaf	Raw	ND	ND	3.18[c]	100.00[b]
	Boiled	ND	ND	4.74[b]	116.66[b]
	Blanched	ND	ND	6.42[ab]	171.67[a]
	Steamed	ND	ND	7.80[a]	215.65[a]
	Microwaved	ND	ND	6.21[ab]	169.88[a]
Spinach	Raw	337.48[b]	100.00[a]	2.34[c]	100.00[ab]
	Boiled	220.14[d]	40.12[c]	3.69[ab]	94.93[ab]
	Blanched	283.96[c]	57.85[b]	3.35[bc]	97.27[ab]
	Steamed	262.78 [cd]	44.75[c]	3.61[ab]	87.70[b]
	Microwaved	499.26[a]	91.10[a]	4.67[a]	121.21[a]
Zucchini	Raw	151.30[bc]	100.00[a]	0.19[c]	100.00[a]
	Boiled	131.82[c]	63.71[b]	0.23[b]	87.09[b]
	Blanched	163.86[b]	87.01[a]	0.23[b]	93.78[ab]

Table 2 continued

Sample	Cooking treatment	Vitamin C		Vitamin K	
		CW[a] (mg/kg)	TR[b] (%)	CW[a] (mg/kg)	TR[b] (%)
	Steamed	199.53[a]	89.24[a]	0.25[b]	90.22[ab]
	Microwaved	205.80[a]	92.73[a]	0.29[a]	101.95[a]

All values are means of duplicate cooking trial and duplicate analysis. Mean values in a row followed by different superscript letters are significantly ($p < 0.05$) different (Duncan's multiple range test)

[a]*CW* cooked weight

[b]TR(%) = (Nc*Gc)/(Nr*Gr)*100, Nc = nutrient contents per gram of sample after cooking, Gc = gram of sample after cooking, Nr = nutrient content per gram of sample before cooking, Gr = gram of sample before cooking

[c]*ND* not determined

vegetables such as broccoli, onion, potatoes and carrots. The effect of cooking on vitamin K has not yet been fully investigated, but increases may be because heat treatment causes vitamin K to be released. Vitamin K is located in the chloroplast in plants and the cooking process may breakdown the plant cell wall, thereby releasing vitamin K and making it available for detection by HPLC. Moreover, vitamin K is relatively heat stable and is thus retained after the cooking process [22]. In contrast, there was no apparent effect of cooking on the vitamin K content of mallow and carrot.

The effect of cooking methods on vitamin E

Vitamin E consists of four tocopherols (α-, β-, γ-, and δ-) and the corresponding tocotrienols (α-, β-, γ-, and δ-) which contain unsaturated side chains. Among the tocochromanol family, α-tocopherol is believed to present the most biological antioxidant activity, mainly attributed to inhibition of membrane lipid peroxidation [23]. In addition, the synergy effect between vitamin C and vitamin E in protecting against lipid peroxidation in liposomes has been reported [24]. The content and true retention of α-tocopherol, γ-tocopherol, and total tocopherol in raw and cooked vegetables are presented in Table 3. In raw vegetables, α-tocopherol was the major tocochromanol with amounts ranging from 0.39 to 92.07 mg/kg of fresh weight. In contrast, a low level of γ-tocopherol was determined in raw broccoli, mallow, crown daisy, perilla leaf, and zucchini, with levels ranging from 0.00 to 5.17 mg/kg of fresh weight. Levels are in agreement with other studies that show green leafy vegetables have higher vitamin E, occurring mainly as α-tocopherol and situated inside chloroplasts [25]. Cooking fresh broccoli, chard, mallow, crown daisy, perilla leaf, spinach, and zucchini lead to a significant increase in α-tocopherol, while cooking potato, sweet potato, and carrot lead to a significant decrease in α-tocopherol. Green leafy or flower vegetables have a higher retention of α-tocopherol than root vegetables, which may be attributed to the increased extractability of α-tocopherol following denaturation of proteins and a complete breakdown of the cell wall in plants which occur as a result of cooking. Moreover, there was a trend toward higher retention of γ-tocopherol in cooked rather than raw vegetables. This high content of vitamin E in cooked samples could be attributed to two reasons: (1) The effect of heat treatment encountered during domestic cooking may cause softening of the tissue by cell disruption in plants and consequently result in the release of vitamin E from the lipids and then become more available for extraction and (2) the heat treatment may also abolish the activity of tocopherol oxidase, which was found in all parts of plant like roots, stems, leaves, flowers and fruits [26]. It has been suggested that oxidizing enzymes maybe involved in the loss of vitamin E during food processing [27]. Plant tissue damage, caused by cutting or mixing could activate oxidizing enzymes involved in the loss of vitamin E due to the collapse of cell compartments [28], but heat treatment could deactivate endogenous oxidative enzymes [29].

The effect of cooking methods on β-carotene

Carotenoids are the precursors of vitamin A and those commonly occurring in nature include α-, β-, and γ-carotene, lycopene, and cryptoxanthin, which are converted to vitamin A in the human body. Among these precursors, β-carotene accounts for more than 90% of total carotenoids in vegetables and plays a crucial role in a major proportion of vitamin A activity [30]. Vitamin A is vital for maintaining the integrity of epithelial tissue growth, proper functioning of the retina and immune system [31]. The content and true retention of β-carotene in raw vegetables and its changes after cooking are shown in Table 3. The β-carotene content of raw vegetables ranged from 0.72 to 42.63 mg/kg of fresh weight, but it was not detected in potato or sweet potato. The β-carotene retention of cooked vegetables was in the range of 40.02–125.37%. It is known that β-carotene extractability may be influenced by cooking, but it can be

Table 3 The contents and true retention of vitamin E and β-carotene in fresh and cooked vegetable

Sample	Cooking treatment	Vitamin E						β-Carotene	
		α-Tocopherol		γ-Tocopherol		Total-tocopherol			
		CW[a] (mg/kg)	TR[b] (%)	CW[a] (mg/kg)	TR[b] (%)	CW[a] (mg/kg)	TR[b] (%)	CW[a] (mg/kg)	TR[b] (%)
Broccoli	Raw	14.87[b]	100.00[b]	2.98[c]	100.00[c]	17.85[b]	100.00[b]	2.56[ab]	100.00[ab]
	Boiled	25.39[a]	166.96[a]	4.18[b]	133.36[b]	29.57[a]	161.16[a]	2.33[bc]	89.40[ab]
	Blanched	25.84[a]	170.19[a]	4.23[b]	137.07[b]	30.07[a]	164.28[a]	2.01[c]	78.23[b]
	Steamed	24.20[a]	161.62[a]	5.17[a]	169.20[a]	29.37[a]	162.34[a]	2.71[ab]	105.93[a]
	Microwaved	23.73[a]	144.59[a]	4.82[ab]	145.36[b]	28.55[a]	144.86[a]	3.02[a]	109.71[a]
Chard	Raw	10.74[d]	100.00[c]	ND[c]	ND	10.74[d]	100.00[c]	15.22[d]	100.00[b]
	Boiled	23.50[a]	136.71[a]	ND	ND	23.50[a]	136.71[a]	30.06[a]	124.22[a]
	Blanched	15.73[c]	114.50[b]	ND	ND	15.73[c]	114.50[b]	21.28[c]	109.36[ab]
	Steamed	15.00[c]	104.31[bc]	ND	ND	15.00[c]	104.31[bc]	25.40[b]	125.37[a]
	Microwaved	17.40[b]	102.80[c]	ND	ND	17.40[b]	102.80[c]	26.78[b]	112.06[a]
Mallow	Raw	6.43[b]	100.00[b]	2.67[c]	100.00[b]	9.11[b]	100.00[b]	34.99[b]	100.00[ab]
	Boiled	10.33[a]	149.86[a]	4.10[b]	149.36[a]	14.43[a]	147.78[a]	41.39[ab]	109.92[ab]
	Blanched	9.81[a]	139.53[a]	4.24[b]	146.05[a]	14.05[a]	141.33[a]	46.42[a]	119.16[a]
	Steamed	10.49[a]	145.34[a]	4.45[b]	148.88[a]	14.94[a]	146.25[a]	33.89[b]	85.61[b]
	Microwaved	10.33[a]	122.94[ab]	4.89[a]	140.50[a]	15.23[a]	128.12[ab]	46.56[a]	101.94[ab]
Potato	Raw	0.56[a]	100.00[a]	ND	ND	0.56[a]	100.00[a]	ND	ND
	Boiled	0.43 [cd]	71.38[c]	ND	ND	0.43 [cd]	71.38[c]	ND	ND
	Blanched	0.50[ab]	84.11[b]	ND	ND	0.50[ab]	84.11[b]	ND	ND
	Steamed	0.39[d]	66.15 [cd]	ND	ND	0.39[d]	66.15 [cd]	ND	ND
	Microwaved	0.47[bc]	59.96[d]	ND	ND	0.47[bc]	59.96[d]	ND	ND
Sweet potato	Raw	5.68[bc]	100.00[a]	ND	ND	5.68[bc]	100.00[a]	ND	ND
	Boiled	5.44[c]	97.18[ab]	ND	ND	5.44[c]	97.18[ab]	ND	ND
	Blanched	5.56[c]	98.24[ab]	ND	ND	5.56[c]	98.24[ab]	ND	ND
	Steamed	6.28[b]	107.59[a]	ND	ND	6.28[b]	107.59[a]	ND	ND
	Microwaved	7.06[a]	88.72[b]	ND	ND	7.06[a]	88.72[b]	ND	ND
Carrot	Raw	3.74[b]	100.00[a]	ND	ND	3.74[b]	100.00[a]	29.38[a]	100.00[a]
	Boiled	3.04[c]	69.46[b]	ND	ND	3.04[c]	69.46[b]	16.23 [cd]	47.36[c]
	Blanched	3.49[bc]	84.26[ab]	ND	ND	3.49[bc]	84.26[ab]	19.02[bc]	58.29[b]
	Steamed	3.98[b]	89.79[a]	ND	ND	3.98[b]	89.79[a]	14.06[d]	40.02[d]
	Microwaved	5.55[a]	86.62[ab]	ND	ND	5.55[a]	86.62[ab]	20.66[b]	40.80[d]
Crown daisy	Raw	3.72[d]	100.00[c]	0.21[c]	100.00[c]	3.93[d]	100.00[c]	7.78[b]	100.00[a]
	Boiled	8.23[ab]	148.40[ab]	0.40[ab]	135.35[bc]	8.63[ab]	147.30[ab]	6.34[b]	57.38[b]
	Blanched	6.98[bc]	149.52[ab]	0.40[ab]	150.33[ab]	7.38[bc]	149.54[ab]	8.06[b]	86.10[ab]
	Steamed	9.01[a]	159.11[a]	0.53[a]	165.77[a]	9.54[a]	159.62[a]	10.67[a]	96.83[a]
	Microwaved	6.17[c]	130.74[b]	0.28[bc]	108.26[c]	6.46[c]	129.60[b]	7.70[b]	83.34[ab]
Perilla leaf	Raw	42.01[c]	100.00[b]	0.89[b]	100.00[c]	42.90[c]	100.00[b]	42.63[a]	100.00[a]
	Boiled	78.29[b]	151.61[a]	1.48[a]	138.00[a]	79.77[b]	156.22[a]	34.26[b]	65.72[c]
	Blanched	70.26[b]	146.89[a]	1.37[a]	134.53[ab]	71.42[b]	146.61[a]	42.74[a]	84.85[b]
	Steamed	92.07[a]	192.44[a]	1.34[a]	130.47[abc]	93.40[a]	191.14[a]	36.23[ab]	72.75[bc]
	Microwaved	74.98[b]	163.86[a]	1.03[b]	104.65[bc]	76.02[b]	162.62[a]	39.03[ab]	81.25[b]
Spinach	Raw	20.03[d]	100.00[b]	ND	ND	20.03[d]	100.00[b]	27.56[d]	100.00[ab]
	Boiled	52.00[b]	158.37[a]	ND	ND	52.00[b]	158.37[a]	46.54[ab]	102.43[ab]
	Blanched	44.82[c]	151.64[a]	ND	ND	44.82[c]	151.64[a]	35.50 [cd]	86.83[b]
	Steamed	57.56[a]	165.42[a]	ND	ND	57.56[a]	165.42[a]	41.18[bc]	84.67[b]
	Microwaved	56.68[a]	174.45[a]	ND	ND	56.68[a]	174.45[a]	51.58[a]	114.67[a]

Table 3 continued

Sample	Cooking treatment	Vitamin E						β-Carotene	
		α-Tocopherol		γ-Tocopherol		Total-tocopherol			
		CW[a] (mg/kg)	TR[b] (%)	CW[a] (mg/kg)	TR[b] (%)	CW[a] (mg/kg)	TR[b] (%)	CW[a] (mg/kg)	TR[b] (%)
Zucchini	Raw	2.99[b]	100.00 [cd]	1.70[b]	100.00[a]	4.69[d]	100.00[ab]	0.72[ab]	100.00[a]
	Boiled	4.74[a]	116.24[a]	2.42[ab]	103.96[a]	7.16[ab]	111.83[a]	0.41[b]	42.13[b]
	Blanched	3.56[b]	96.25[d]	1.89[ab]	90.56[a]	5.46[c]	93.74[b]	0.66[ab]	74.33[a]
	Steamed	4.60[a]	105.01[bc]	2.26[ab]	91.73[a]	6.86[b]	99.89[ab]	0.87[a]	82.47[a]
	Microwaved	4.93[a]	112.17[ab]	2.71[a]	105.88[a]	7.64[a]	110.98[a]	0.82[a]	77.21[a]

All values are means of duplicate cooking trial and duplicate analysis. Mean values in a row followed by different superscript letters are significantly ($p < 0.05$) different (Duncan's multiple range test)

[a]*CW* cooked weight

[b]TR(%) = (Nc*Gc)/(Nr*Gr)*100, Nc = nutrient contents per gram of sample after cooking, Gc = gram of sample after cooking, Nr = nutrient content per gram of sample before cooking, Gr = gram of sample before cooking

[c]*ND* not determined

Table 4 Precision and accuracy for vitamins in broccoli

Type of vitamin	Parameter	Precision		Accuracy[a]
		Repeatability[b]	Reproducibility[c]	Recovery (%)
Vitamin C	Mean[d]	682.66	665.05	105.15
	SD	15.82	19.35	3.81
	CV (%)	2.32	2.91	3.63
α-Tocopherol	Mean	4.26	5.24	98.68
	SD	0.12	0.19	1.80
	CV (%)	2.78	3.56	1.82
γ-Tocopherol	Mean	0.95	1.10	116.55
	SD	0.04	0.07	4.74
	CV (%)	4.20	6.07	4.07
Vitamin K	Mean	1.42	1.46	99.88
	SD	0.04	0.07	3.00
	CV (%)	3.07	4.69	3.01
β-Carotene	Mean	0.52	0.54	102.27
	SD	0.01	0.03	1.18
	CV (%)	2.14	5.48	1.15

[a]Accuracy is a measure of the closeness of the analytical result to the true value determined by analyzing a spiked sample

[b]Repeatability was evaluated using five independent analyses of replicate sample performed on a given day

[c]Reproducibility was evaluated using five independent analyses of replicate sample performed on a different day

[d]Concentration of vitamins expressed as mg/100 g as dry weight basis

enhanced or not [18]. The present study showed that β-carotene retention of cooked broccoli, chard, mallow, and spinach was higher than in the corresponding raw samples except for several cooking processes. Carotenoids including β-carotene are found in the chloroplasts of all green plant tissues, where they occur in the photosynthetic pigment-protein complexes, which have an inhibitory effect on extractability of β-carotene from the vegetable matrix. Cooking of foods could increase the extraction of carotenoids by softeningplant walls and disrupting carotenoid-protein complexes [32, 33]. It is assumed that an increased extractability may be associated with improved bioavailability. In contrast, β-carotene retention of cooked carrot, crown daisy, perilla leaf, and zucchini was lower than the raw samples. Our results agree with those of a previous study, which reported to a decrease in β-carotene in carrots after boiling [34]. This low thermal stability of β-carotene observed for carrot could be due to the different

intracellular location of β-carotene. Carotene in carrots was found to be located in crystalline chromoplasts with membranes rich in polar lipids. Thermal treatment of carrots could cause the alteration of the physical state of carotenes. In particular, blanching allowed the solubilization of carotenes by cellular lipids [35]. Moreover, the difference in the β-carotene retention of cooked vegetables might be attributed to the extent of loss of carotene caused by dripping during the cooking process.

Method validation

The analytical methods used in this study were validated in terms of accuracy and precision for all tested vitamins. The precision and accuracy for vitamins in broccoli are shown in Table 4. Accuracy of the method was measured based on recovery (%). The recoveries for vitamin C, α-tocopherol, γ-tocopherol, vitamin K and β-carotene were 105.15 ± 3.81, 98.68 ± 1.80, 116.55 ± 4.74, 99.88 ± 3.00 and 102.27 ± 1.18, respectively. Precision of the method was assessed based on the repeatability (% coefficient of variation, %CV) and reproducibility (%CV). The repeatability and reproducibility was less than 5%, except for γ-tocopherol and β-carotene. The higher %CV for γ-tocopherol and β-carotene might have been caused by the lower content found in broccoli. The proposed analytical method was validated in terms of specificity for vitamin E and vitamin K. In general, the results from the method validation are satisfactory.

In conclusion, the present study examined the effect of different cooking methods including blanching, boiling, microwaving, and steaming, on the content and true retention of β-carotene, ascorbic acid, vitamins E and vitamin K in vegetables. Cooking may cause changes to the contents of vitamins and it depends on the type of vegetables and the method of cooking methods. Therefore, further research is needed to optimize cooking procedures to enhance retention of vitamins.

Acknowledgements This study was supported by the Rural Development Administration (Project Number PJ010838).

References

1. Gerald F, Combs JR, James P McClung. The vitamins: fundamental aspects in nutrition and health. 5th ed. Academic Press, MA, USA. pp. 3–6 (2016)
2. Cobley JN, MaHardy H, Morton JP, Nikolaidis MG, Close GL. Influence of vitamin C and vitamin E on redox signaling: Implications for exercise adaptations. Free Radic. Biol. Med. 84: 65–76 (2015)
3. Fuchs J, Kern H. Modulation of UV-light-induced skin inflammation by D-alpha-tocopherol and L-ascorbic acid: a clinical study using solar simulated radiation. Free Radic. Biol. Med. 25: 1006–1012 (1998)
4. 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 ER, Tangrea JA, Teppo L, Askin FB, Taskinen E, Erozan Y, Greenwald P, Huttunen JK. Alpha-Tocopherol and beta-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 Inst. 88: 1560–1570 (1996)
5. Heinonen OP, Albanes D, Virtamo J, Taylor PR, Huttunen JK, Hartman AM, Haapakoski J, Malila N, Rautalahti M, Ripatti S, Maenpaa H, Teerenhovi L, Koss L, Virolainen M, Edwards BK. Prostate cancer and supplementation with alpha-tocopherol and beta-carotene: incidence and mortality in a controlled trial. J. Natl. Cancer Inst. 90: 440–446 (1998)
6. Palozza P, Krinsky NI. Beta-Carotene and alpha-tocopherol are synergistic antioxidants. Arch. Biochem. Biophys. 297: 184–187 (1992)
7. Cheung CL, Sahni S, Cheung BM, Sing CW, Wong IC. Vitamin K intake and mortality in people with chronic kidney disease from NHANES III. Clin. Nutr. 34: 235–240 (2015)
8. Karmas E, Harris RS. Nutritional evaluation of food processing. 3th ed. Van Nostrand Reinhold Company, NY, USA. pp. 360–365 (1998)
9. Hu FB. Dietary pattern analysis: a new direction in nutritional epidemiology. Curr. Opin. Lipidol. 13: 3–9 (2002)
10. Zhong X, Dolan KD, Almenar E. Effect of steamable bag microwaving versus traditional cooking methods on nutritional preservation and physical properties of frozen vegetables: A case study on broccoli (*Brassica oleracea*). Innov. Food Sci. Emerg. Tech. 31: 116–122 (2015)
11. Dos Reis LC, de Oliveira VR, Hagen ME, Jablonski A, Flores SH, de Oliveira Rios A. Effect of cooking on the concentration of bioactive compounds in broccoli (*Brassica oleracea* var. Avenger) and cauliflower (*Brassica oleracea* var. Alphina F1) grown in an organic system. Food Chem. 172: 770–777 (2015)
12. Tian J, Chen J, Lv F, Chen S, Chen J, Liu D, Ye X. Domestic cooking methods affect the phytochemical composition and antioxidant activity of purple-fleshed potatoes. Food Chem. 197: 1264–1270 (2016)
13. Xu F, Zheng Y, Yang Z, Cao S, Shao X, Wang H. Domestic cooking methods affect the nutritional quality of red cabbage. Food Chem. 161: 162–167 (2014)
14. Lee J, Suknark K, Kluvitse Y, Phillips RD, Eitenmiller RR. Rapid liquid chromatographic assay of vitamin E and retinyl palmitate in extruded weaning foods. J. Food Sci. 64: 968–972 (1999)
15. Jakob E, Elmadfa I. Application of a simplified HPLC assay for the determination of phylloquinone (vitamin K_1) in animal and plant food items. Food Chem. 56: 87–97 (1996)
16. United States Department of Agriculture, USDA Table of Nutrient Retention Factors, Release 6 (2007) Available from: https://www.ars.usda.gov. Accessed May. 1, 2017.
17. Phillips KM, Tarragó-Trani MT, Gebhardt SE, Exler J, Patterson KY, Haytowitz DB, Pehrsson PR, Holden JM. Stability of vitamin C in frozen raw fruit and vegetable homogenates. J. Food Compos. Anal. 23: 253–259 (2010)
18. Bureau S, Mouhoubi S, Touloumet L, Garcia C, Moreau F, Bédouet V, Renard C MGC. Are folates, carotenoids and vitamin C affected by cooking? Four domestic procedures are compared on a large diversity of frozen vegetables. LWT-Food Sci. Technol. 64: 735–741 (2015)
19. Manna P, Kalita J. Beneficial role of vitamin K supplementation on insulin sensitivity, glucose metabolism, and the reduced risk of type 2 diabetes: A review. Nutrition 32: 732–739 (2016)

20. Booth SL, Suttie JW. Dietary intake and adequacy of vitamin K. J. Nutr. 128: 785–788 (1998)
21. Damon M, Zhang N, Haytowitz D, Booth S. Phylloquinone (vitamin K1) content of vegetables. J. Food Compos. Anal. 18: 751–758 (2005)
22. Scognamiglio M, D'Abrosca B, Pacifico S, Isidori M, Fiorentino A. Fat-soluble vitamins. Vol. II, pp. 568–569. In: handbook of food analysis-3rd ed. Nollet LML and Toldrá F (ed). CRC Press Inc., Baca Raton, FL, USA (2015)
23. Cruz R, Casal S. Validation of a fast and accurate chromatographic method for detailed quantification of vitamin E in green leafy vegetables. Food Chem. 141: 1175–1180 (2013).
24. Barclay LRC, Locke SJ, MacNeil JM. The autoxidation of unsaturated lipids in micelles. Synergism of inhibitors vitamins C and E. Can. J. Chem. 61:1288–1290 (1983)
25. Bramley PM, Kafatos A, Kelly FJ, Manios Y, Roxborough HE, Schuch W, Sheehy PJA, Wagner KH. Review vitamin E. J. Sci. Food Agric. 80: 913–938 (2000)
26. Murillo E, Plumpton SE, Gaunt JK. The properties and distribution of α-tocopherol oxidase in plants. Biochem. Soc. Trans. 4: 486–487 (1976)
27. Knecht K, Sandfuchs K, Kulling SE, Bunzel D. Tocopherol and tocotrienol analysis in raw and cooked vegetables: a validated method with emphasis on sample preparation. Food Chem. 169: 20–27 (2015)
28. Lee TC, Ho CT. Bioactive compounds in foods: Effects of processing and storage, American Chemical Society, Washington DC, USA. pp. 34–43 (2002)
29. Choi Y, Lee SM, Chun J, Lee HB, Lee J. Influence of heat treatment on the antioxidant activities and polyphenolic compounds of Shiitake (*Lentinus edoes*) mushroom. Food Chem. 99: 381–387 (2006)
30. Gayathri GN, Platel K, Prakash J, Srinivasan K. Influence of antioxidant spices on the retention of β-carotene in vegetables during domestic cooking processes. Food Chem. 84: 35–43 (2003)
31. Nzamwita M, Duodu KG, Minnaar A. Stability of beta-carotene during baking of orange-fleshed sweet potato-wheat composite bread and estimated contribution to vitamin A requirements. Food Chem. 228: 85–90 (2017)
32. Bernhardt S, Schlich E. Impact of different cooking methods on food quality: Retention of lipophilic vitamins in fresh and frozen vegetables. J. Food Eng. 77: 327–333 (2006)
33. Castenmiller JJ, West CE, Linssen JP, van het Hof KH, Voragen AG. The food matrix of spinach is a limiting factor in determining the bioavailability of beta-carotene and to a lesser extent of lutein in humnas. J. Ntr. 129: 345–355 (1999)
34. Mazzeo T, N'Dri D, Chiavaro E, Visconti A, Fogliano V, Pellegrini N. Effect of two cooking procedures on phytochemical compounds, total antioxidant capacity and colour of selected frozen vegetable. Food Chem. 128: 627–633 (2011)
35. Mara M, Stuparic M, Schieber A, Carle R. Effect of thermal processing on trans-cis-isomerization of β-carotene in carrot juices and carotene-containing preparations. Food Chem. 83:609–617 (2003)