pISSN 1229-1153 / eISSN 2465-9223 *J. Food Hyg. Saf.*
Vol. 37, No. 5, pp. 297~305 (2022)
https://doi.org/10.13103/JFHS.2022.37.5.297

Journal of Food Hygiene and Safety
Available online at http://www.foodhygiene.or.kr

Determination and Validation of Synthetic Antioxidants in Processed Foods Distributed in Korea

Hyeon-Ju Park[1], Eunbin Seo[1], Jin-Wook Park[1], Choong-In Yun[2], Young-Jun Kim[1]*

[1]*Department of Food Science and Technology, Seoul National University of Science and Technology*
[2]*Research Institute of Food and Biotechnology, Seoul National University of Science and Technology, Seoul, Korea*

(Received August 01, 2022/Revised August 10, 2022/Accepted September 02, 2022)

ABSTRACT - Antioxidants are food additives that extend the shelf life of food products by preventing lipid rancidity caused by active oxygen. They can either be naturally-derived or manufactured synthetically via chemical synthesis. In this study, method validation of five synthetic antioxidants, namely butylated hydroxyanisole, butylated hydroxytoluene, tertiary butylhydroquinone, propyl gallate, and disodium ethylenediaminetetraacetic acid, was performed using a high performance liquid chromatography–ultraviolet visible detector, and the method applicability was evaluated by analyzing foods containing antioxidants. The coefficient of determination (R^2) average was 0.9997, while the limit of detection and limit of quantification were 0.02–0.53 and 0.07–1.61 mg/kg, respectively. The intra and inter-day accuracies and precisions were 83.2±0.7%–98.7±2.1% and 0.1%–5.7% RSD, respectively. Inter-laboratory validation for accuracy and precision was conducted using the Food Analysis Performance Assessment Scheme quality control material. The results satisfied the guidelines presented by the AOAC International. In addition, the expanded uncertainty was less than 16%, as recommended by CODEX. Consequently, to enhance public health safety, the results of this study can be used as basis data for evaluating the intake of synthetic antioxidants and assessing their risks in Korea.

Key words: Food additives, Synthetic antioxidants, High-performance liquid chromatography, Method validation, Measurement uncertainty

With advances in the manufacturing and processing technology in the food industry, today's food has become more diverse in types and forms[1)]. As a result, the consumption of food additives has risen with the increase in processed and preserved foods[2)]. Food additives are necessary and unavoidable in food development and quality preservation, but despite rigorous legal regulations and standards, more and more consumers are skeptical about the widespread application and safety of food additives and try to avoid foods containing food additives or eat foods with fewer food additives, which are perceived as healthier. The use of food additives has several advantages, such as extending shelf life as well as providing a variety of food choices, and consumers are aware of these benefits[3)]. In Korea, the Food Sanitation Act was first implemented in 1962, which is the basis for regulating food and food additives, and as of 2019, 618 food additives were allowed[4)].

Excessive amounts of active oxygen change the structure of proteins, nucleic acids, and lipids, and lipid oxidation produces off-flavors or undesirable chemical compositions, such as aldehydes, ketones, and organic acids. Antioxidants extend the shelf life of foods by preventing fat rancidity and pigment discoloration, among other deteriorative reactions of oxygen-sensitive foodstuffs. In general, antioxidants may be synthetic or natural but have the common function of interfering with the formation of active oxygen that causes oxidation[5)]. The compounds commonly found in natural antioxidants are phenols, polyphenols, flavonoids, cinnamic acid derivatives, tocopherols, and organic acids and are generally present in berries, roots, leaves, and flowers of various fruits, vegetables, spices, and herbs[6)]. However, the use of natural antioxidants that meet the requirements of the food industry often encounters difficulties because of the difficulty of extraction and insufficient stability[7)].

Synthetic antioxidants are often more stable and active than natural antioxidants and are widely used in the food industry due to the diversity of raw materials and economic

*Correspondence to: Young-Jun Kim, Department of Food Science and Technology, Seoul National University of Science and Technology, Seoul 01811, Korea
Tel: +82-2-970-6734, Fax: +82-2-970-9736
E-mail: kimyj@seoultech.ac.kr

advantages[8]. Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), *tert*-butylhydroquinone (TBHQ), and propyl gallate (PG) are examples of synthetic antioxidants incorporated into food products[9]. These synthetic antioxidants are intentionally added to prevent and delay the oxidation of lipid components during the processing of fat, oil, and fat-containing foods and have a history of use of more than 50 years[10,11].

Disodium ethylenediaminetetraacetic acid (EDTA·2 Na), the sodium salt of EDTA, is widely used as a food additive to inhibit the catalytic oxidation of lipids by metal ions because of the ease with which it forms stable complexes with a variety of metal ions[12,13]. Metal ion chelation is one of the general antioxidant methods, and EDTA usually binds to a metal cation as a hexadentate chelating agent through its two amines and four carboxylates.

As the most common synthetic antioxidants incorporated in foodstuffs, BHA, BHT, TBHQ, and PG are added to animal fats and vegetable oil. These may be used alone or in combination. The European Food Safety Authority (EFSA) established the Acceptable Daily Intake (ADI) levels of BHA, BHT, TBHQ, PG, and EDTA·2 Na as 1.0, 0.25, 0.7, 0.5, and 1.9 mg/kg body weight (bw)/day, respectively[14]. For the same components, the Joint Food and Agricultural Organization of the United Nations/World Health Organization (FAO/WHO) Expert Committee on Food Additives (JECFA) set ADI levels of 0.5, 0.3, 0.7, 1.4, and 1.9 mg/kg bw/day[15]. In Korea, these antioxidants can be used in foods at a concentration range of 0.035–1.00 g/kg[4]. In the United States, the European Union (EU), and other countries, these may be used alone or in combination at up to 0.01% or 0.02%. In accordance with the recommendations of international organizations, the use of synthetic antioxidants, such as BHA, BHT, TBHQ, and PG, and chelating agents, such as EDTA·2 Na, is regulated by legal authorities in a limited number of foods, with maximum limits in each case[16].

Various analytical techniques have been used for the detection of food additives. Some common techniques are high-performance liquid chromatography with ultraviolet detection (HPLC-UVD)[17], high-performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS)[18], gas chromatography with flame ionization detection (GC-FID)[19], and gas chromatography with mass spectrometry (GC-MS)[20]. Previous studies related to the method development[21] and validation[22,23] of food additives have also been conducted, and risk assessment and intake assessment of food additives have been published[24].

In this study, the status of four synthetic antioxidants (BHA, BHT, TBHQ, and PG) and EDTA·2 Na among food additives permitted in Korea were identified. Moreover, according to the Food Code[25] and Food Additives Code[4], commercial products labeled with any of the five additives were analyzed for method validation and quantitative analysis using HPLC-UVD.

Materials and Methods

Sample collections

For monitoring, foods containing antioxidants as additives were purchased from supermarkets, convenience stores, and online markets in the year 2019. A total of 213 items were purchased: 99 snacks, 20 fried noodles, 14 chewing gum, 11 breads, 38 sauces, 11 processed peanuts or nuts, 10 processed fruits and vegetables, and 10 mayonnaises.

Reagents and materials

The BHA, BHT, TBHQ, and PG standards used in this study were products of Toronto Research Chemicals (North York, Canada). Acetic acid (Sigma-Aldrich, St. Louis. MO, USA) was used for the preparation of the mobile phase. Water and acetonitrile were supplied by J.T. Baker (Phillipsburg, NJ, USA). 2-Propanol was used for standard solution preparation, and hexane was used for extraction; both were HPLC-grade and procured from Fisher Chemicals (Middlesex County, MA, USA).

The EDTA·2 Na standard, and iron chloride hexahydrate used for the preparation of the standard solution, were purchased from Toronto Research Chemicals. Hydrochloric acid (HCl), methylene chloride used for extraction, and tetrabutylammonium hydroxide solution and acetic acid used for the preparation of the mobile phase were purchased from Sigma-Aldrich. Ethanol was sourced from J.T. Baker.

Analytical instrument

For the simultaneous analysis of four antioxidants (BHA, BHT, TBHQ, and PG), an Agilent 1100 Series HPLC (Agilent Technologies, Inc., Santa Clara, CA, USA) was used with UVD. The column was an Imtakt Unison US-C18 (150 mm × 4.6 mm, 5 μm), and the detection wavelength was set to 280 nm.

For the analysis of EDTA·2 Na, the Agilent 1100 Series HPLC (Agilent Technologies, Inc.) was equipped with a Shiseido C18 UG120 column (150 mm × 4.6 mm, 5 μm), and the detection wavelength was set to 254 nm. The detailed HPLC instrument conditions are shown in Table 1.

Preparation of standard solutions

Standard solutions for each of the four synthetic antioxidants were prepared by precisely taking 10.2 mg of BHA, BHT, and TBHQ and 4.1 mg of PG in 100-mL volumetric flasks, and the volume was completed with 2-propanol. The standard solutions were prepared at concentrations of 1, 5, 20,

Table 1. HPLC conditions for analysis of synthetic antioxidants

Compound	BHA, BHT, TBHQ, PG			EDTA·2 Na
Instrument	HPLC(Agilent 1100 series)			HPLC(Agilent 1100 series)
Column	Imtakt Unison US-C18 (150 mm × 4.6 mm, 5 μm)			Capcell pak C18 UG120 (150 mm × 4.6 mm, 5 μm)
Detector	UVD (280 nm)			UVD (254 nm)
Mobile phase	A: 0.2% Acetic acid B: 90% Acetonitrile			0.01M Tetrabutylammonium hydroxide solution : Ethanol (19 : 1)
	Time(min)	A(%)	B(%)	
	0	85	15	
	5	85	15	
	18	10	90	
	23	10	90	
	25	85	15	
	30	85	15	
	36	0	100	
Flow rate	1.0 mL/min			1.0 mL/min
Injection volume	10 μL			10 μL
Column temp.	25°C			25°C

50, and 100 mg/kg by diluting the respective standard stock solutions with 2-propanol.

The standard solution for EDTA·2 Na was prepared by precisely taking 114.3 mg of EDTA·2 Na in a 100-mL volumetric flask, and the volume was completed with distilled water. Then, 0.02, 0.1, 0.2, and 0.5 mL of the standard stock solution were dispensed into separate 10-mL volumetric flasks, followed by 5 mL of 0.01 M iron (III) chloride solution and dilution with distilled water to prepare concentrations of 2, 10, 20, and 50 mg/kg.

Sample preparation

The sample preparation for the four synthetic antioxidants (BHA, BHT, TBHQ, and PG) was carried out using different pretreatment methods depending on the food type. For oils and fats, hexane (5 mL) was added three times each to 5 g of the liquid oil sample in a beaker and combined in a separatory funnel. Extraction was carried out using 50 mL of hexane-saturated acetonitrile three times. The lower layer of the extract was transferred to a 250-mL round-bottomed flask and concentrated under reduced pressure to 3-4 mL in a water bath at 35°C. The concentrate was transferred to a 10-mL volumetric flask, the round-bottomed flask was washed with 2-propanol, and the rinse solution was added to the volumetric flask. The volume was completed with 2-propanol and then filtered through a 0.45-μm syringe filter (Sartorius Minisart® RC, Sartorius Co., Gottingen, Germany). In the case of a solid fat sample, the sample was melted at 60°C, and 2.5 g was processed in the same way as the liquid oil sample.

For snacks, hexane (100 mL) was added to a mixture of 5 g of the homogenized sample and 15 g of anhydrous sodium sulfate in a 250-mL tube and mixed for 5 min. The mixture was filtered through a 25-μm membrane filter (Qualitative Filter Paper No. 4, Whatman, Maidstone, UK) into a 500-mL round-bottomed flask. Hexane (100 mL) was added to the residue, the extraction was repeated, and the filtrates were combined. The pooled filtrate and washings (three times with 5 mL hexane) were concentrated to about 20 mL and then transferred to a 125-mL separatory funnel. After extraction with 50 mL of saturated acetonitrile three times each, the lower layer of the extract was poured into a 250-mL round-bottomed flask and concentrated under reduced pressure to 3-4 mL in a water bath at 35°C. The concentrate was transferred to a 10-mL volumetric flask, the round-bottomed flask was rinsed with 2-propanol, and the rinse solution was added to the volumetric flask. The volume was completed with 2-propanol and then filtered through a 0.45-μm syringe filter (Sartorius Minisart® RC, Sartorius Co.)[25].

For EDTA·2 Na, Water and methylene chloride (10 mL each) were added to 10 g of the homogenized sample. After mixing well so that the layer disappeared, extraction was performed by shaking at 300 rpm for 5 min using a shaker (SI-600R, JeioTech Co., Daejeon, Korea). The extract was centrifuged at 4000 rpm for 15 min (Ohaus® Frontier™ 5706, Ohaus Corp., Parsippany, NJ, USA), and only the supernatant consisting of water was transferred to a 20-mL flask. For high-fat samples, such as mayonnaise, after

extraction by shaking for 5 min, 10 mL of methylene chloride was added, and the mixture was shaken for 5 min and centrifuged. The water layer was taken, water was added to the remaining part, and the extraction (shaking for 5 min) was repeated, followed by centrifugation (4,000 rpm for 15 min). The supernatant was collected in a 20-mL flask, diluted with water, mixed 1:1 with a 0.01 M iron (III) chloride solution, and filtered through a 0.45-μm syringe filter (Sartorius Minisart® RC, Sartorius Co.)[25].

Method validation

In this study, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, and accuracy were calculated according to the International Council for Harmonisation (ICH) guideline[26]. Based on this, the method validation was analyzed. The standard stock solution was diluted and analyzed six times using HPLC-UVD. A calibration curve was prepared by calculating the peak area for each concentration, and the coefficient of determination (R^2) was calculated to evaluate linearity. To calculate LOD and LOQ, a calibration curve was prepared by repeating the three lowest concentrations of the calibration curve three times. The standard deviation of the *y*-intercept (σ) and the mean of the slope (S) were used in the following equations to derive the LOD and LOQ:

$$LOD = \frac{3.3 \times \sigma}{S},\ LOQ = \frac{10 \times \sigma}{S}$$

Precision indicates the proximity between the measured values and is expressed as the relative standard deviation (RSD) of the repeated measurement results. Accuracy refers to the closeness of the analysis result to the true value and is expressed as a recovery rate (%). For the recovery test, three concentrations of four synthetic antioxidants (BHA, BHT, and TBHQ: 5, 20, and 50 mg/kg, PG: 2, 8, and 20 mg/kg) were added to vegetable oil and snack that did not contain the four synthetic antioxidants. Three concentrations of EDTA·2 Na (2, 10, and 25 mg/kg) were added to canned food and mayonnaise that did not contain EDTA·2 Na. For intra-day precision, the recovery rate experiment was performed six times within a day, and three concentrations were repeated three times for 3 days for inter-day precision. The Horwitz ratio (HorRat value) is a parameter indicating the applicability of the analysis method through the precision value of the experiment and can be calculated as follow. RSD_R is the reproducibility (%) that can be calculated from the experimental result value, and $PRSD_R$ denotes the RSD value predicted from the Horwitz equation. If the ratio is less than 1, the precision of the experiment is significant; the experimentally applicable range is between 0.5 and 2.0[27].

$$\text{HorRat} = \frac{RSD_R}{PRSD_R}$$

Inter-laboratory validation

Inter-laboratory validation was conducted at four different analytical laboratories (Lab. 1, Lab. 2, Lab. 3, and Lab. 4) and was evaluated by comparing the analysis results using the same sample and the same analytical method in each laboratory. For the experiment, the Food Analysis Performance Assessment Scheme (FAPAS) quality control (QC) material (T2015QC) was analyzed for BHA, BHT, and PG. The sample was analyzed three times, and the average contents were calculated. Then, the contents were compared to the assigned values (%), and the %RSD values were measured.

Measurement uncertainty assessment

Uncertainty is defined in the ISO/IEC 17025:2017 International Standards for Quality Management Systems in Pharmaceutical Laboratories as the degree of dispersion of values that can reasonably be attributed to a measured value. The uncertainty value can be estimated by comparing the inter-laboratory data, and the reliability of the measurement can be obtained based on the comparison result. To conduct the uncertainty estimation, the mathematical processing and statistical methods recommended by EURACHEM (A Focus for Analytical Chemistry in Europe)[28] were used. The measurement uncertainty was estimated for the standard stock solution preparation (u*SSS*), sample preparation (u*SP*), calibration curve (u*Cal*.), and repeated measurement of samples (u*RP*). Moreover, the expanded uncertainty (U*c*) was estimated using the coverage factor (k) of 2 at the 95% confidence level.

Results and Discussion

Method validation

Specificity was validated by confirming that there were no interfering substances in the peak retention times of the synthetic antioxidants in each sample. The retention times of BHA, BHT, TBHQ, PG, and EDTA·2 Na were 18.0, 24.7, 14.8, 12.4, and 4.9 min, respectively. Calibration curves were constructed by repeated (six times) analysis of BHA, BHT, TBHQ, and PG at five concentrations within the range of 1.0–100 mg/L, and EDTA·2 Na at four concentrations within the range of 2.0–50.0 mg/L. The coefficient of determination (R^2) was 0.9989–0.9999, and it satisfied the minimum standards of the U.S. Food and Drug Administration (FDA) of ≥ 0.995[29]. LOD and LOQ were 0.02–0.53 and 0.07–1.61 mg/kg, respectively. The calibration parameter results are detailed in Table 2.

Intra- and inter-day accuracy and precision results are shown in Table 3. The results of the intra- and inter-day

Table 2. Calibration parameter results of synthetic antioxidants

Parameters	BHA	BHT	TBHQ	PG	EDTA·2 Na
Range of calibration (mg/L)	1.0–100	1.0–100	1.0–100	0.4–40	2.0 - 50
Coefficient of determination (R^2)	0.9999	0.9999	0.9989	0.9999	0.9999
Slope (±S.D)	7.49±0.02	4.92±0.01	7.72±0.16	26.16±0.09	16.15±0.05
Intercept (±S.D)	0.50±0.35	0.11±0.23	-3.46±1.97	-3.71±0.42	0.99±1.29
LOD (mg/kg)[1)]	0.05	0.26	0.13	0.02	0.53
LOQ (mg/kg)[2)]	0.14	0.79	0.39	0.07	1.61

[1)]Limit of detection.
[2)]Limit of quantification.

Table 3. Validation results of accuracy, precision, and expanded measurement uncertainty of synthetic antioxidants

Compound	Sample	Added standard (mg/kg)	Intra-day[1)]			Inter-day[2)]			Expanded uncertainty (%)
			Accuracy (%)[3)]	Precision (%RSD)	HorRat (r)[4)]	Accuracy (%)	Precision (%RSD)	HorRat (r)[5)]	
BHA	Vegetable oil	5	93.5±1.9	2.0	0.32	93.7±1.8	1.9	0.30	9.88
		20	95.5±1.8	1.9	0.38	95.6±1.6	1.7	0.33	3.10
		50	96.7±3.4	3.5	0.80	97.1±3.0	3.1	0.70	3.29
	Snack	5	96.9±2.4	2.4	0.39	96.6±2.2	2.3	0.37	9.60
		20	96.8±1.3	1.3	0.26	97.0±1.1	1.1	0.22	2.86
		50	98.7±2.1	2.1	0.48	98.3±2.1	2.1	0.47	2.34
BHT	Vegetable oil	5	89.6±2.7	3.0	0.48	89.6±2.9	3.3	0.53	10.53
		20	88.0±1.0	1.1	0.22	88.0±1.0	1.2	0.24	3.00
		50	89.3±3.0	3.4	0.77	89.6±3.0	3.3	0.75	3.23
	Snack	5	94.3±2.3	2.5	0.40	93.8±2.2	2.4	0.38	9.94
		20	89.0±2.4	2.7	0.53	89.0±2.1	2.4	0.47	3.58
		50	92.4±2.8	3.1	0.69	92.6±2.3	2.5	0.56	2.98
TBHQ	Vegetable oil	5	97.2±2.7	2.8	0.45	97.2±2.3	2.4	0.38	14.22
		20	95.8±3.1	3.2	0.63	96.3±3.0	3.2	0.63	6.68
		50	97.8±4.4	4.5	1.02	97.6±4.5	4.6	1.04	4.57
	Snack	5	89.6±5.7	6.4	1.02	89.7±5.1	5.7	0.91	14.83
		20	93.1±0.9	1.0	0.20	92.9±1.0	1.0	0.20	6.37
		50	95.4±2.9	3.0	0.68	95.7±2.9	3.0	0.68	3.67
PG	Vegetable oil	2	95.0±2.9	3.1	0.43	94.4±2.6	2.8	0.39	11.74
		8	92.9±2.2	2.4	0.42	93.0±2.2	2.4	0.41	4.08
		20	96.3±4.1	4.3	0.85	96.4±4.0	4.2	0.83	3.96
	Snack	2	85.5±3.1	3.7	0.51	86.2±3.3	3.8	0.53	12.65
		8	91.0±0.7	0.8	0.14	91.1±0.7	0.7	0.12	3.71
		20	96.1±2.0	2.1	0.41	96.4±1.8	1.8	0.36	2.51
EDTA·2 Na	Canned food	2	83.2±0.7	0.9	0.12	87.0±2.4	2.7	0.38	10.63
		10	85.7±0.1	0.1	0.01	87.5±1.8	2.0	0.35	3.84
		50	89.2±0.1	0.1	0.03	87.9±1.0	1.2	0.27	1.88
	Mayonnaise	2	94.6±0.8	0.8	0.11	94.6±0.9	0.9	0.13	9.80
		10	91.5±0.2	0.2	0.04	94.0±1.8	1.9	0.34	3.63
		50	97.3±0.2	0.2	0.05	94.7±1.7	1.8	0.41	1.81

[1)]Analysis was conducted six time/day.
[2)]Analysis was conducted three times on three days.
[3)]Average±SD.
[4)]HorRat ratio for intra-day repeatability.
[5)]HorRat ratio for inter-day repeatability.

accuracy, evaluated by spiking various foods with the synthetic antioxidants (BHA, BHT, and TBHQ: 5, 20, and 50 mg/kg; PG: 2, 8, and 20 mg/kg; EDTA·2 Na: 2, 1, and 50 mg/kg) were 83.2±0.7–97.8±4.4 and 86.2±3.3–98.3±2.1%, respectively. Intra- and inter-day precision with the same analyte were 0.1–6.4 and 0.7–5.7 %RSD, respectively. The results were within the acceptable values established by the AOAC guideline[30]. In addition, the HorRat values were less than 1.02 (intra-day) and less than 1.04 (inter-day). These results satisfied the recommended ranges[27].

Measurement uncertainty

The measurement uncertainty was estimated by the recovery test conducted in this study. The uncertainty value related to u*SSS*, u*SP*, u*Cal*., and u*RP* were considered. As shown in Table 3, the expanded uncertainty was 3.00–14.22% in vegetable oil, 2.34–14.83% in snacks, 1.88–10.63% in canned food, and 1.81–9.80% in mayonnaise. The results were within the acceptable range of the CODEX standard (≤ 16%)[31]. In most samples, the expanded uncertainty decreased with the increasing concentration of the synthetic antioxidant. The results of contributions of measurement uncertainty to the expanded uncertainty were shown in Fig. 1.

Inter-laboratory validation

An oil sample (FAPAS QC Material, T20153QC) containing BHA, BHT, and PG was used for inter-laboratory validation. The result is shown as recovery±SD (%), and the average of each laboratory's result and precision (%RSD) are provided in Table 4. Recovery was 102.7±0.44% for BHA, 102.2±0.55% for BHT, and 105.3±0.40 % for PG, and the corresponding precision was 0.43, 0.54, and 0.38 %RSD, respectively. These results satisfied the AOAC guidelines[30].

Application

The monitoring results for the four synthetic antioxidants (BHA, BHT, TBHQ, PG), and EDTA·2 Na in the selected 213 foods distributed in Korea are shown in Table 5. If the

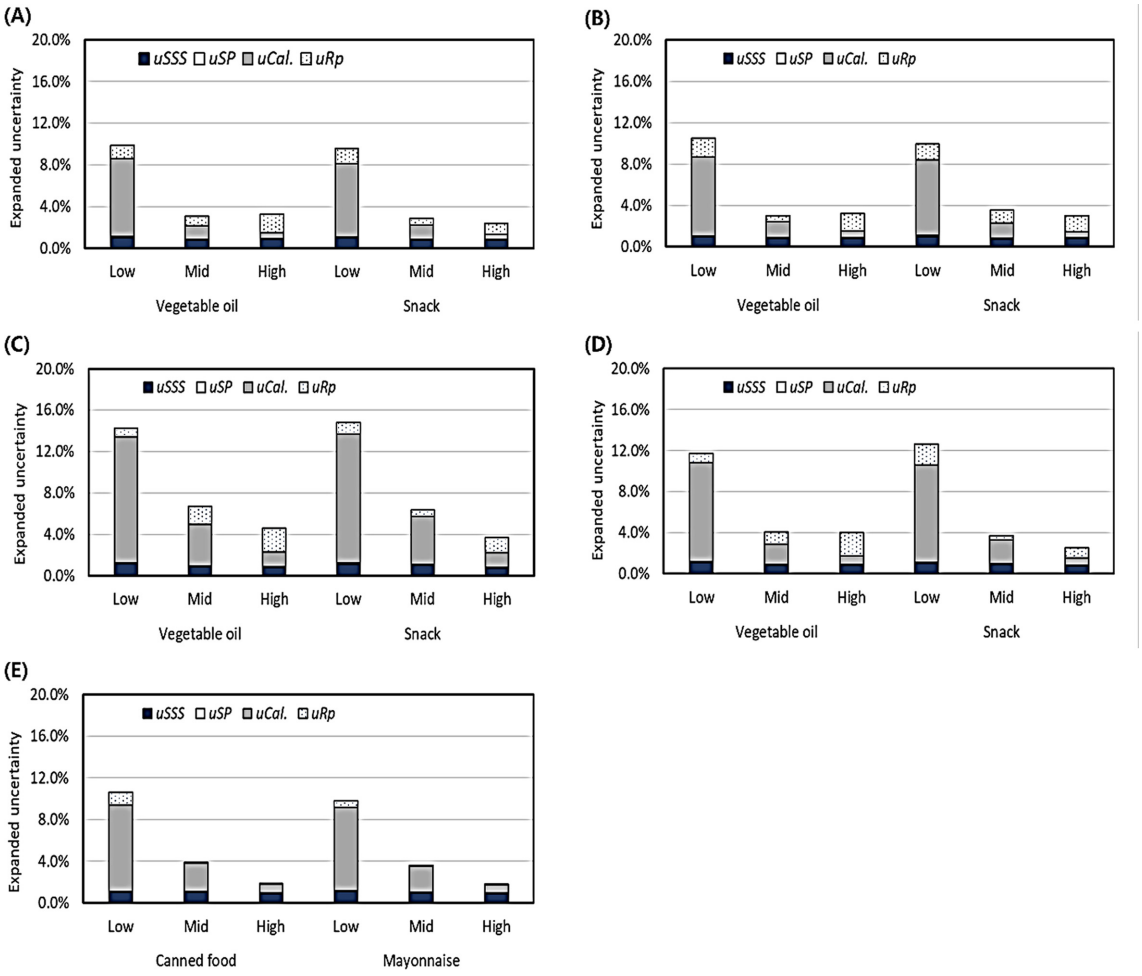

Fig. 1. Contributions of measurement uncertainty to the expanded uncertainty of five synthetic antioxidants spiked in vegetable oil, snack, canned food, and mayonnaise. (**A**) BHA, (**B**) BHT, (**C**) TBHQ, (**D**) PG, and (**E**) EDTA·2 Na

Table 4. Inter-laboratory reproducibility of recovery of synthetic antioxidants

Compound	Assigned value (mg/kg)	Recovery±SD (%)				Average±SD (%)	%RSD
		Lab 1	Lab 2	Lab 3	Lab 4		
BHA	76.6	105.4±0.20	103.9±0.3	97.8±1.07	103.9±0.2	102.7±0.44	0.43
BHT	41.3	101.6±0.61	101.7±0.64	102.4±0.48	102.9±0.48	102.2±0.55	0.54
PG	55.7	105.7±0.41	104.3±0.82	105.3±0.1	106.0±0.27	105.3±0.40	0.38

*Food Analysis Performance Assessment Scheme (FAPAS) quality control (QC) material (T2015QC) was used.

Table 5. Concentration (mg/kg) and the range of synthetic antioxidants in foods

Compound	Food code	No. of sample	Concentration (mg/kg)		
			Range	Overall average (mg/kg)	Positive average (mg/kg)
BHA	Snack	26	N.D–18.26	3.41	4.93
	Oil-fried noodle	10	N.D–8.16	3.54	5.90
BHT	Snack	27	N.D–44.21	4.40	9.14
	Chewing gum	14	30.98–153.78	64.91	64.91
	Bread	11	N.D–2.43	0.22	2.43
TBHQ	Snack	26	N.D–9.22	3.22	3.81
	Oil-fried noodle	10	N.D–5.76	1.35	3.37
PG	Snack	10	N.D–0.1	0.1	0.1
EDTA·2 Na	Sauce	38	N.D–91.9	44.56	48.37
	Processed Peanut or Nut Products	11	N.D–101.1	27.65	50.68
	Snack	10	N.D–14.9	3.40	11.33
	Processed fruit/vegetable product	10	N.D–73.8	13.85	69.25
	Mayonnaise	10	46.3–61.2	52.66	52.66

analyte was not detected, it was expressed as not detected (N.D.). The concentration ranges were N.D.–18.26 mg/kg of BHA in snacks and oil-fried noodles, N.D.–153.78 mg/kg of BHT in snacks, chewing gums, and breads, and N.D.–9.22 mg/kg of TBHQ in snacks and oil-fried noodles. PG was analyzed in snacks, and it was detected at low levels of N.D.–0.1 mg/kg. The analysis of sauces, processed peanut or nut products, snacks, processed fruit/vegetable products, and mayonnaises for EDTA·2 Na revealed concentrations in the range of N.D.–101.1 mg/kg. As a result, chewing gum with 153.78 mg/kg of BHT was the food with the highest level of any of the five additives. This content complied with the food additive standards (≤ 400 mg/kg)[4].

Similar to the experiments conducted in this study using HPLC-UVD, a previous study of the antioxidants in edible oils and fats using HPLC-MS/MS found that all of them were acceptable within the limit of 200 mg/kg[18]. In the EU and the United States, antioxidants are permitted on foods at levels of 100–200 mg/kg, and according to a previous study, the contents found satisfied the standard for oils and fats, butter, margarine, and similar products[32]. It was confirmed that the use of antioxidants was thoroughly managed. Consequently, to enhance public health safety, the results of this study can be used as basic data for evaluating the intake of synthetic antioxidants and its risk assessments in Korea.

Acknowledgement

This research was supported by a grant from Ministry of Food and Drug Safety in 2019.

국문요약

산화방지제는 활성 산소에 의한 지질 산패를 방지하여 식품의 보존성을 증대하는 식품첨가물이다. 본 연구에서는 합성 산화방지제 5종 (Butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT), Tertiary butylhydroquinone (TBHQ), Propyl gallate (PG), Disodium ethylenediaminetetraacetic acid (EDTA·2 Na))을 이용하여 HPLC-UVD 분석법에 대한 유효성 검증 및 합성 산화방지제가 표시된 실제 식품 속 함량 분석을 진행하였다. 직선성(R^2)은 평균 0.9997, 검출한계(Limit of detection, LOD)와 정량한계(Limit of quantification, LOQ)는 각각 0.02–0.53과 0.07–1.61 mg/kg으로 산출하였

다. 일내 및 일간 정확성과 정밀성 산출을 위하여 합성 산화방지제 4종은 콩기름과 과자, EDTA는 통조림과 마요네스를 이용하였다. 정확성과 정밀성 결과, 일내와 일간 각각 83.2±0.7–97.8±4.4와 86.2±3.3–98.3±2.1 및 0.1–6.4와 0.7–5.7 %RSD로 산출되었다. 또한 FAPAS QC material을 이용하여 실험실간 정확성 및 정밀성 검증을 진행하였으며 이는 AOAC가 제시한 가이드라인에 적합함을 확인하였다. 측정불확도 역시 CODEX에서 제시한 범위인 16% 이하인 것을 확인하였다. 따라서 본 연구를 통하여 국민건강의 안전을 제고하기 위한 국내 산화방지제의 섭취량 및 위해성 평가를 위한 기초자료로 활용될 수 있을 것으로 사료된다.

Conflict of interests

The authors declare no potential conflict of interest.

ORCID

Hyeon-Ju Park https://orcid.org/0000-0002-0744-1912
Eunbin Seo https://orcid.org/0000-0002-9610-4837
Jin-Wook Park https://orcid.org/0000-0003-4257-2667
Choong-In Yun https://orcid.org/0000-0002-4305-678X
Young-Jun Kim https://orcid.org/0000-0001-7232-6008

References

1. Wongnen, C., Ruzzama, N., Chaijan, M., Cheong, L.Z., Panpipat, W., Glochidion wallichianum Leaf Extract as a Natural Antioxidant in Sausage Model System. *Foods*, **11**, 1547 (2022).
2. Kim, H.Y., Lee, Y.J., Hong, K.H., Ha, S.C., Ahn, M.S., Jo, J.S., Kim, K.S., Intake of food additives in foods by total diet. *Korean J. Food Sci. Technol.*, **30**, 767-774 (1998).
3. Kaptan, B., Kayısoglu, S., Consumers' attitude towards food additives. *Am. J. Food Sci. & Nutr. Res.*, **2**(2), 21-25 (2015).
4. Ministry of Food and Drug Safety. Food Additives Code "Chapter 2. Use Level of each food additive"
5. Karovičová, J., Šimko, P., Determination of synthetic phenolic antioxidants in food by high-performance liquid chromatography. *J. Chromatogr. A*, **882**(1-2), 271-281 (2000).
6. Rodil, R., Quintana, J.B., Cela, R., Oxidation of synthetic phenolic antioxidants during water chlorination. *J. Hazard. Mater.* , **199**, 73-81 (2012).
7. Saad, B., Sing, Y.Y., Nawi, M.A., Hashim, N., Aldi, A.S.M., Saleh, M.I., Ahmad, K., Determination of synthetic phenolic antioxidants in food items using reversed-phase HPLC. *Food Chem.*, **105**(1), 389-394 (2007).
8. Xiu-Qin, L., Chao, J., Yan-Yan, S., Min-Li, Y., Xiao-Gang, C., Analysis of synthetic antioxidants and preservatives in edible vegetable oil by HPLC/TOF-MS. *Food Chem.*, **113**, 692-700 (2009).
9. Xu, X., Liu, A., Hu, S., Ares, I., Martínez-Larrañaga, M.R., Wang, X., Martínez, M.A., Synthetic phenolic antioxidants: Metabolism, hazards and mechanism of action. *Food Chem.*, **353**, 129488 (2021).
10. Formanek, Z., Kerry, J.P., Higgins, F.M., Buckley, D.J., Morrissey, P.A., Farkas, J., Addition of synthetic and natural antioxidants to α-tocopheryl acetate supplemented beef patties: effects of antioxidants and packaging on lipid oxidation. *Meat Sci.*, **58**, 337-341 (2001).
11. McCarthy, T.L., Kerry, J.P., Kerry, J.F., Lynch, P.B., Buckley, D.J., Evaluation of the antioxidant potential of natural food/plant extracts as compared with synthetic antioxidants and vitamin E in raw and cooked pork patties. *Meat Sci.*, **58**, 45-52 (2001).
12. Yu, Z., Lu, L., Lu, L., Pan, L., Qiu, X., Tang, Y., Development and antioxidation of metal ion chelating packaging film. *Food Packag. Shelf Life*, **32**, 100846 (2022).
13. Kristinova, V., Aaneby, J., Mozuraityte, R., Storrø, I., Rustad, T., The effect of dietary antioxidants on iron-mediated lipid peroxidation in marine emulsions studied by measurement of dissolved oxygen consumption. *Eur. J. Lipid Sci. Technol*., **116**, 857-871 (2014).
14. European Food Safety Authority. Scientific Opinion on the reevaluation of butylated hydroxyanisole-BHA (E 320) as a food additive. Panel on Food Additives and Nutrient Sources added to Food (ANS). *EFSA Journal*, **9**, 2392 (2011).
15. FAO (Food and Agriculture Oraganization of the United Nations), https://www.fao.org/home/en/
16. André, C., Castanheira, I., Cruz, J.M., Paseiro, P., Sanches-Silva, A., Analytical strategies to evaluate antioxidants in food: a review. *Trends Food Sci. Technol.*, **21**, 229-246 (2010).
17. Andrikopoulos, N.K., Brueschweiler, H., Felber, H., Taeschler, C., HPLC analysis of phenolic antioxidants, tocopherols and triglycerides. *J. Am. Oil Chem. Soc.*, **68**, 359-364 (1991).
18. Kim, J.M., Choi, S.H., Shin, G.H., Lee, J.H., Kang, S.R., Lee, K.Y., Lee, O.H., Method validation and measurement uncertainty for the simultaneous determination of synthetic phenolic antioxidants in edible oils commonly consumed in Korea. *Food Chem.*, **213**, 19-25 (2016).
19. Yang, M.H., Lin, H.J., Choong, Y.M., A rapid gas chromatographic method for direct determination of BHA, BHT and TBHQ in edible oils and fats. *Food Res. Int*, **35**, 627-633 (2002).
20. Guo, L., Xie, M.Y., Yan, A.P., Wan, Y.Q., Wu, Y.M., Simultaneous determination of five synthetic antioxidants in edible vegetable oil by GC-MS. *Anal. Bioanal. Chem*, **386**, 1881-1887 (2006).
21. Kang, H.H., Yun, C.I., Lee, G., Shin, J.W., Kim, Y.J., Development and validation of an analytical method for betanine and isobetanine in processed food products labeled with beet red. *J. Food Hyg. Saf*, **36**(5), 376-381 (2021).
22. Yang, H.J., Seo, E., Yun, C.I., Kim, Y.J., Determination of sodium alginate in processed food products distributed in Korea. *J. Food Hyg. Saf*, **36**, 474-480 (2021).

23. Seo, E., Lim, S., Yun, C.I., Shin, J.W., Kim, Y.J., Validation and measurement uncertainty of HPLC-UV method for quercetin quantification in various foods. *Korean J. Food Sci. Technol.*, **53**, 682-687 (2021).
24. Kang, H.H., Yun, C.I., Choi, S., Oh, K.S., Kim, Y.J., Occurrence and risk characterization of non-nutritive sweeteners in selected food products from Korea. *Food Sci. Biotech.*, **31**(1), 37-48 (2022).
25. Ministry of Food and Drug Safety. Food Code "Chapter 8. General Test Methods"
26. ICH Harmonised Tripartite Guideline. 2005. Validation of analytical procedures: text and methodology Q2 (R1). International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use, San Diego, CA, USA. pp. 1-13.
27. Horwitz, W., Albert, R., The Horwitz ratio (HorRat): A useful index of method performance with respect to precision. *J. AOAC Int* , **89**(4), 1095-1109 (2006).
28. Ellison, S.L., Williams, A. (2012). Quantifying uncertainty in analytical measurement.
29. US Food and Drug Administration. (2014). Methods, Method Verification and Validation.
30. AOAC. Official methods of analysis of AOAC International. AOAC International, Rockville, MD, USA. (2016)
31. CODEX Alimentarius Commission. (2008). Guidance on measurement uncertainty and uncertainty of sampling guidance on measurement uncertainty, CX. MAS 08/29/9. Joint FAO/WHO Food Standards Programme CODEX Committee on Methods of Analysis and Sampling. Budapest, Hungary.
32. Shasha, D., Magogo, C., Dzomba, P. Reversed phase HPLC-UV quantitation of BHA, BHT and TBHQ in food items sold in Bindura supermarkets, Zimbabwe. *Int. Res. J. Pure Appl. Chem*., **4**(5), 578-584 (2014).