

## Comparison of the chemical compositions and nutritive values of various pumpkin (*Cucurbitaceae*) species and parts

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### Abstract

Pumpkins have considerable variation in nutrient contents depending on the cultivation environment, species, or part. In this study, the general chemical compositions and some bioactive components, such as tocopherols, carotenoids, and  $\beta$ -sitosterol, were analyzed in three major species of pumpkin (*Cucurbitaceae pepo*, *C. moschata*, and *C. maxima*) grown in Korea and also in three parts (peel, flesh, and seed) of each pumpkin species. *C. maxima* had significantly more carbohydrate, protein, fat, and fiber than *C. pepo* or *C. moschata* ( $P < 0.05$ ). The moisture content as well as the amino acid and arginine contents in all parts of the pumpkin was highest in *C. pepo*. The major fatty acids in the seeds were palmitic, stearic, oleic, and linoleic acids. *C. pepo* and *C. moschata* seeds had significantly more  $\gamma$ -tocopherol than *C. maxima*, whose seeds had the highest  $\beta$ -carotene content. *C. pepo* seeds had significantly more  $\beta$ -sitosterol than the others. Nutrient compositions differed considerably among the pumpkin species and parts. These results will be useful in updating the nutrient compositions of pumpkin in the Korean food composition database. Additional analyses of various pumpkins grown in different years and in different areas of Korea are needed.

**Key Words:** Pumpkins, macronutrients, tocopherols, carotenoids,  $\beta$ -sitosterol

### Introduction

Pumpkins are gourd squashes of the genus *Cucurbita* and the family *Cucurbitaceae*. The pumpkin species available include *C. pepo* (called “Kuksuhobak” in Korean), *C. moschata* (“neulgeunhobak”), and *C. maxima* (“danhobak”). These three species are cultivated worldwide and have high production yields [1].

Pumpkins are cooked and consumed in many ways, and most parts of the pumpkin are edible, from the fleshy shell to the seeds. In Korea, pumpkin flesh is consumed in soups and juices, or it is incorporated into various foods, such as rice cakes, candies, and breads. In the US and Canada, pumpkin is a Halloween and Thanksgiving staple. Pumpkin seeds and pumpkin seed oil are also commonly consumed in some countries.

Pumpkins have long been used for traditional medicine in many countries, such as China, Argentina, India, Mexico, Brazil, and Korea, since pumpkin flesh and seeds are rich not only in proteins, antioxidant vitamins, such as carotenoids and tocopherols [2], and minerals, but low in fat and calories.  $\beta$ -carotene reduces skin damage from the sun and acts as an anti-inflammatory agent.  $\alpha$ -carotene is thought to slow the aging process, reduce the risk

of developing cataracts, and prevent tumor growth. Vitamin E (tocopherols) protects the cell from oxidative damage by preventing the oxidation of unsaturated fatty acids in cell membrane. Pumpkin seeds, often eaten as a snack, are a good source of zinc, polyunsaturated fatty acids [3,4], and phytosterols (e.g.  $\beta$ -sitosterol) [1,5], which can prevent chronic diseases. Recent studies have reported that pumpkin can benefit the treatment of benign prostate hyperplasia, because of its high  $\beta$ -sitosterol content [6-9].  $\beta$ -Sitosterol has been indicated to reduce blood cholesterol and to decrease risks of certain types of cancers.

The most frequently consumed *Cucurbita* species in Korea are *C. moschata* and *C. maxima*, whereas *C. pepo* consumption is relatively low. Thus, there is limited research regarding *C. pepo* in Korea. Other countries, however, including the US and Canada consume more *C. pepo* than other species. In 2006, the National Rural Living Science Institute in Korea updated their food composition tables [10]. The Korean food composition tables include 4 types of pumpkins (mature pumpkin, young pumpkin, zucchini squash, and sweet pumpkin), mainly *C. moschata* and *C. maxima* [10]. Some nutrient contents in *C. pepo* are also reported, but the amino acid, fatty acid, vitamin E, and carotenoid

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contents in *C. pepo* are not available. Currently, there is limited research analyzing the nutrients in *C. pepo* grown in Korea, and the nutrients in the different parts of each pumpkin species. Because the nutrient composition of pumpkins will differ depending on their origins and cultivation environments [11-15], it may be important to know the nutritional profiles of the various pumpkin species grown in Korea and of the various parts of these pumpkins. Moreover, *C. pepo* harvest and consumption are gradually increasing in Korea. Therefore, this study determined the general nutrient composition, including amino acids, fatty acids, and specific bioactive nutrients, such as tocopherols, carotenoids, and  $\beta$ -sitosterol, of 3 pumpkin species grown and consumed in Korea (*C. pepo*, *C. moschata*, and *C. maxima*) and 3 different parts (peel, flesh, and seed) of each species.

## Materials and Methods

### Sample preparation

*C. pepo* was obtained from a local farm (Gunsan, Korea). *C. moschata* (Naju, Korea) and *C. maxima* (Kochang, Korea) were purchased from agricultural product joint markets in Kwangju, Korea. Over 20 pumpkins of each species were purchased. All samples were harvested and collected in the fall of 2008. The samples were divided into 3 parts: peel, flesh, and seed. Samples were freeze-dried, mixed using a hand blender (PHILIPS HR-1372, Koninklijke Philips Electronics N.V., Amsterdam, Netherlands), and stored at  $-70^{\circ}\text{C}$  until analyzed. All samples in this study were analyzed in triplicate.

### Materials

An amino acid standard solution (AA-S-18) was purchased from Fluka Ltd. (Buchs, Switzerland). A fatty acid 37 component methyl ester mix was obtained from Supelco<sup>TM</sup> (Bellefonte, PA, USA).  $\alpha$ - and  $\gamma$ -tocopherol,  $\beta$ -carotene,  $\beta$ -cryptoxanthin, and  $\beta$ -sitosterol standards were obtained from Sigma Chemical Co. (St Louis, MO, USA).

High-performance liquid chromatography (HPLC) grade hexane (JT Baker, Deventer, Holland), tetrahydrofuran (THF, Acros Organics Co., Geel, Belgium), methanol (JT Baker, Deventer, Holland), and acetonitrile (JT Baker, Deventer, Holland) were used. Triethylamine (Fisher Scientific Ltd., Loughborough, UK), dichloromethane (Acros Organics Co., Geel, Belgium), and N,O-Bis (trimethylsilyl) trifluoroacetamide (BHT, Acros Organics Co.) were purchased. All other reagents used were analytical grade.

### Chemical composition

Protein was analyzed using the macro-Kjeldahl method (AOAC 984.13) using a Foss Kjeltac 2300 automatic analyzer (Foss

Tecator AB, Höganäs, Sweden) [16]. Crude fat was analyzed by AOAC method 945.16 with ether as a solvent [16]. Ash was determined by a muffle furnace set at  $550^{\circ}\text{C}$  (AOAC 942.05) [16]. Moisture content was determined using AOAC 930.15 oven drying method at  $105^{\circ}\text{C}$  overnight [16]. Total carbohydrate contents were calculated by  $100 - (\text{g moisture} + \text{g protein} + \text{g fat} + \text{g ash})$  [17].

### Amino acid analysis

Amino acids were measured in hydrolysates using a Sykam-S433D amino acid analyzer (Sykam GmbH, Fürstfeldbruck, Germany). Hydrolysates were prepared as described by Moore and Stein [18] and modified by Mohammed and Yagoub [19]. Ninhydrin solution and an eluent buffer (solvent A: pH 3.45 and solvent B: pH 10.85) were delivered simultaneously into a high temperature reactor coil (16 m long) at a flow rate of 0.7 mL/min. The buffer/ninhydrin mixture was heated in the reactor to  $130^{\circ}\text{C}$  for 2 minutes to accelerate the amino acid reaction with ninhydrin. The reaction products were detected with 570 nm and 440 nm light on a dual channel photometer. The amino acid contents were calculated from the areas of standards obtained from the integrator, and are expressed as percentages.

### Fatty acid analysis

Dried samples were extracted with chloroform:methanol (2:1, v/v) according to the method of Folch *et al.* [20]. Solid and non-lipid material were removed, then the solvent was evaporated under nitrogen gas. Fatty acid methyl ester was prepared by methylating the total lipids, as described by Joseph and Ackman [21]. Methyl esters were separated by gas chromatography (GC), (Varian 3400 capillary GC with a flame ionization detector, Varian, Walnut Creek, CA, USA and SP-2560, 100 m  $\times$  0.25 mm i.d., Supelco Inc., Bellefonte, PA, USA) under the following conditions. The detector temperature was  $280^{\circ}\text{C}$ , the injection port temperature was  $250^{\circ}\text{C}$ , and the column temperature was  $180^{\circ}\text{C}$ . Carrier gas (hydrogen) flow was 1 mL/min with a nitrogen flow of 30 mL/min. The split ratio was 50:1 and samples (1  $\mu\text{L}$ ) were injected in triplicate. To identify each fatty acid, each retention time was compared with the standard (Supelco 37 fatty acid methyl esters).

### Tocopherol and carotenoid analysis

Tocopherols and carotenoids were extracted from pumpkin seeds using a method modified from Kim *et al.* [22], and using HPLC (Gilson 351 HPLC system, Gilson, Villiers le Bel, France) with a 151 UV/VIS detector and a C18 column (250  $\times$  4.6 mm i.d., 5  $\mu\text{m}$ , GraceSmart<sup>TM</sup>, Deerfield, USA). The mobile phase was 40 mL of water (containing triethylamine [500  $\mu\text{L}$ ] and ammonium acetate [0.4 g]), 60 mL of methanol (containing BHT [1 g L<sup>-1</sup>]), 800 mL of acetonitrile, and 100 mL THF. The flow

rate was 1.0 mL/min, and the column temperature was 24°C. Tocopherols and carotenoids were detected at 297 nm and 450 nm, respectively. Tocopherols and carotenoids were quantified using calibration curves obtained with each standard alone and mixed.

### $\beta$ -Sitosterol analysis

Two grams of pumpkin seeds were hydrolyzed with 6 M HCl as described by Toivo *et al.* [23]. Dried extracts were saponified as described by Maguire *et al.* [24]. The hexane layer was dried under nitrogen, redissolved in 200  $\mu$ L ethanol, and stored at -20°C for HPLC analysis on a Gilson HPLC system (Gilson, Villiers le Bel, France) with a Luna C8(2) column (250  $\times$  4.6 mm i.d., 5  $\mu$ m, Phenomenex, Cheshire, UK). The mobile phase was 100% acetonitrile, flow rate was 1.2 mL/min, and the column temperature was 24°C.  $\beta$ -sitosterol was detected at 208 nm using a UV detector.

### Statistical analysis

All statistical analyses were performed using SPSS 15.0 (SPSS, Inc., Chicago, USA). In order to determine the differences in nutrient contents among species, one-way ANOVA tests were performed, followed by post-hoc test (Duncan's multiple range test) to compare means. A *P* value <0.05 was considered significant. Data are presented as mean  $\pm$  standard deviation (SD).

## Results

### Chemical compositions

Table 1 shows the chemical compositions each pumpkin species. The contents of The flesh of *C. maxima*, *C. pepo*, and *C. moschata* contained 26.23  $\pm$  0.20 g carbohydrate /kg raw weight, 42.39  $\pm$  0.84 g/kg, and 133.53  $\pm$  1.44 g/kg, respectively. *C. maxima* had significantly more carbohydrates in the flesh and peel than *C. pepo* and *C. moschata*. *C. maxima* had significantly more protein in the flesh (11.31  $\pm$  0.95 g/kg raw weight) and peel (16.54  $\pm$  2.69 g/kg raw weight) than *C. pepo* and *C. moschata* (*P* < 0.05). *C. pepo* had significantly more protein in the seeds (308.8  $\pm$  12.01 g/kg raw weight) than *C. maxima* (274.85  $\pm$  10.04 g/kg raw weight), (*P* < 0.05). The flesh of *C. pepo* and *C. moschata* had a small amount of fat (0.55  $\pm$  0.14 and 0.89  $\pm$  0.11 g/kg raw weight, respectively). The peel of *C. pepo* and *C. moschata* had similar amounts of fat (4.71  $\pm$  0.69 and 6.59  $\pm$  0.41 g/kg raw weight, respectively). *C. maxima* seeds had significantly more fat (524.34  $\pm$  1.32 g/kg raw weight), (*P* < 0.05) than *C. pepo* or *C. moschata* (439.88  $\pm$  2.88 and 456.76  $\pm$  11.66 g/kg raw weight, respectively). The flesh and seeds of *C. pepo* had significantly lower fiber and ash contents than *C.*

**Table 1.** Chemical compositions (g/kg raw weight) of pumpkins (*Cucurbitaceae*) by species and by part<sup>1)</sup>

Nutrients	Part	Species		
		<i>C. pepo</i>	<i>C. moschata</i>	<i>C. maxima</i>
Carbohydrate	Flesh	26.23 $\pm$ 0.20 <sup>a</sup>	43.39 $\pm$ 0.84 <sup>b</sup>	133.53 $\pm$ 1.44 <sup>c</sup>
	Peel	43.76 $\pm$ 0.74 <sup>a</sup>	96.29 $\pm$ 1.11 <sup>b</sup>	206.78 $\pm$ 3.25 <sup>c</sup>
	Seed	122.20 $\pm$ 7.47 <sup>a</sup>	140.19 $\pm$ 7.60 <sup>b</sup>	129.08 $\pm$ 8.25 <sup>ab</sup>
Protein	Flesh	2.08 $\pm$ 0.11 <sup>a</sup>	3.05 $\pm$ 0.65 <sup>a</sup>	11.31 $\pm$ 0.95 <sup>b</sup>
	Peel	9.25 $\pm$ 0.12 <sup>a</sup>	11.30 $\pm$ 0.99 <sup>a</sup>	16.54 $\pm$ 2.69 <sup>b</sup>
	Seed	308.83 $\pm$ 12.06 <sup>b</sup>	298.11 $\pm$ 14.75 <sup>ab</sup>	274.85 $\pm$ 10.04 <sup>a</sup>
Fat	Flesh	0.55 $\pm$ 0.14 <sup>a</sup>	0.89 $\pm$ 0.11 <sup>b</sup>	4.20 $\pm$ 0.23 <sup>c</sup>
	Peel	4.71 $\pm$ 0.69 <sup>a</sup>	6.59 $\pm$ 0.41 <sup>b</sup>	8.69 $\pm$ 0.99 <sup>c</sup>
	Seed	439.88 $\pm$ 2.88 <sup>a</sup>	456.76 $\pm$ 11.66 <sup>b</sup>	524.34 $\pm$ 1.32 <sup>c</sup>
Fiber	Flesh	3.72 $\pm$ 0.02 <sup>a</sup>	7.41 $\pm$ 0.07 <sup>b</sup>	10.88 $\pm$ 0.35 <sup>c</sup>
	Peel	12.28 $\pm$ 0.15 <sup>a</sup>	34.28 $\pm$ 1.37 <sup>c</sup>	22.35 $\pm$ 0.01 <sup>b</sup>
	Seed	148.42 $\pm$ 0.55 <sup>b</sup>	108.51 $\pm$ 8.36 <sup>a</sup>	161.54 $\pm$ 6.79 <sup>c</sup>
Ash	Flesh	3.44 $\pm$ 0.04 <sup>a</sup>	10.36 $\pm$ 0.01 <sup>b</sup>	10.53 $\pm$ 0.11 <sup>c</sup>
	Peel	6.30 $\pm$ 0.06 <sup>a</sup>	13.96 $\pm$ 0.16 <sup>c</sup>	11.20 $\pm$ 0.64 <sup>b</sup>
	Seed	55.02 $\pm$ 1.00 <sup>c</sup>	53.15 $\pm$ 0.20 <sup>b</sup>	44.22 $\pm$ 0.36 <sup>a</sup>
Moisture	Flesh	967.70 $\pm$ 0.15 <sup>c</sup>	942.31 $\pm$ 0.08 <sup>b</sup>	840.43 $\pm$ 0.17 <sup>a</sup>
	Peel	935.98 $\pm$ 0.27 <sup>c</sup>	871.86 $\pm$ 0.09 <sup>b</sup>	756.79 $\pm$ 0.44 <sup>a</sup>
	Seed	74.06 $\pm$ 0.91 <sup>c</sup>	51.79 $\pm$ 6.04 <sup>b</sup>	27.51 $\pm$ 0.21 <sup>a</sup>

<sup>1)</sup> Values are mean  $\pm$  SD. Different superscript letters within a row indicate significant differences by Duncan's multiple range test (*P* < 0.05).

*moschata* or *C. maxima* (*P* < 0.05). All parts of *C. pepo* had the highest moisture content, and *C. maxima* the lowest.

### Amino acids

The amino acid compositions are presented in Table 2. Except for aspartic acid, the flesh and peel of *C. maxima* had higher amino acid contents than the two species. In the seeds *C. pepo* had the highest amino acid concentrations. Pumpkin seeds were contained all 9 essential amino acids. The arginine content in *C. pepo* seeds (63.99  $\pm$  0.88 mg/kg raw weight) was much higher than in *C. moschata* (7.03  $\pm$  0.58 mg/kg raw weight) or *C. maxima* (8.69  $\pm$  0.97 mg/kg raw weight). Glycine was not detected in the flesh of *C. pepo*, whereas *C. moschata* and *C. maxima* contained small amounts (0.05  $\pm$  0.01 and 0.12  $\pm$  0.01 mg/kg raw weight, respectively). Methionine was not detected in the flesh of *C. pepo* or *C. moschata*, but *C. maxima* contained a small amount (0.11  $\pm$  0.00 mg/kg raw weight).

### Fatty acids

Table 3 shows the fatty acid compositions in pumpkin seeds. Seven kinds of fatty acids in *C. pepo*, 4 fatty acids in *C. moschata*, and 10 fatty acids in *C. maxima* were detected in this study. The seeds were 18.62-20.11% saturated fatty acid, 14.90-32.40% monounsaturated fatty acid (MUFA), and 35.72-56.84% polyunsaturated acids (PUFA). *C. pepo* and *C. moschata* seeds contained similar amounts of oleic acid (*C. pepo*: 32.40  $\pm$  0.56% fat, *C. moschata*: 31.34  $\pm$  0.12% fat) and linoleic acid (*C. pepo*:

**Table 2.** Amino acids concentrations (mg/kg raw weight) in pumpkins (*Cucurbitaceae*) by species and by part<sup>1)</sup>

Amino acids	Part	Species		
		<i>C. pepo</i>	<i>C. moschata</i>	<i>C. maxima</i>
Alanine	Flesh	0.12 ± 0.03 <sup>a</sup>	0.22 ± 0.01 <sup>b</sup>	0.77 ± 0.02 <sup>c</sup>
	Peel	0.73 ± 0.00 <sup>a</sup>	0.56 ± 0.00 <sup>a</sup>	1.52 ± 0.26 <sup>b</sup>
	Seed	17.76 ± 0.03 <sup>c</sup>	7.43 ± 1.01 <sup>a</sup>	10.16 ± 0.64 <sup>b</sup>
Arginine	Flesh	0.54 ± 0.11 <sup>b</sup>	0.07 ± 0.01 <sup>a</sup>	1.11 ± 0.03 <sup>c</sup>
	Peel	1.12 ± 0.05 <sup>c</sup>	0.23 ± 0.01 <sup>a</sup>	0.60 ± 0.09 <sup>b</sup>
	Seed	63.99 ± 0.88 <sup>c</sup>	7.03 ± 0.58 <sup>a</sup>	8.69 ± 0.97 <sup>a</sup>
Aspartic acid	Flesh	0.44 ± 0.05 <sup>a</sup>	2.83 ± 0.10 <sup>c</sup>	2.21 ± 0.14 <sup>b</sup>
	Peel	1.57 ± 0.05 <sup>a</sup>	2.82 ± 0.06 <sup>c</sup>	2.39 ± 0.36 <sup>b</sup>
	Seed	29.95 ± 0.25 <sup>c</sup>	15.31 ± 1.00 <sup>a</sup>	20.41 ± 1.08 <sup>b</sup>
Glutamic acid	Flesh	0.94 ± 0.17 <sup>a</sup>	1.03 ± 0.05 <sup>a</sup>	4.32 ± 0.29 <sup>b</sup>
	Peel	1.98 ± 0.09 <sup>a</sup>	2.22 ± 0.01 <sup>a</sup>	4.10 ± 0.67 <sup>b</sup>
	Seed	60.26 ± 0.04 <sup>b</sup>	42.94 ± 3.06 <sup>a</sup>	48.94 ± 3.55 <sup>a</sup>
Glycine	Flesh	ND <sup>2)</sup>	0.05 ± 0.01 <sup>b</sup>	0.12 ± 0.01 <sup>c</sup>
	Peel	0.23 ± 0.02 <sup>a</sup>	0.88 ± 0.04 <sup>c</sup>	0.52 ± 0.13 <sup>b</sup>
	Seed	18.70 ± 0.36 <sup>b</sup>	12.97 ± 1.63 <sup>a</sup>	16.47 ± 1.35 <sup>b</sup>
Histidine	Flesh	0.19 ± 0.04 <sup>a</sup>	0.30 ± 0.04 <sup>b</sup>	1.11 ± 0.05 <sup>c</sup>
	Peel	0.78 ± 0.02 <sup>a</sup>	1.02 ± 0.04 <sup>a</sup>	1.58 ± 0.23 <sup>b</sup>
	Seed	18.37 ± 0.08 <sup>b</sup>	11.74 ± 0.89 <sup>a</sup>	16.51 ± 2.21 <sup>b</sup>
Isoleucine	Flesh	0.15 ± 0.03 <sup>a</sup>	0.11 ± 0.02 <sup>a</sup>	0.71 ± 0.06 <sup>b</sup>
	Peel	0.59 ± 0.03 <sup>a</sup>	0.50 ± 0.01 <sup>a</sup>	1.10 ± 0.12 <sup>b</sup>
	Seed	13.96 ± 0.74 <sup>c</sup>	8.50 ± 1.10 <sup>a</sup>	10.89 ± 1.09 <sup>b</sup>
Leucine	Flesh	0.10 ± 0.03 <sup>a</sup>	0.15 ± 0.02 <sup>a</sup>	0.90 ± 0.07 <sup>b</sup>
	Peel	0.63 ± 0.03 <sup>a</sup>	0.88 ± 0.01 <sup>a</sup>	1.70 ± 0.23 <sup>b</sup>
	Seed	24.14 ± 0.96 <sup>b</sup>	16.51 ± 1.75 <sup>a</sup>	19.11 ± 1.91 <sup>a</sup>
Lysine	Flesh	0.03 ± 0.01 <sup>a</sup>	0.07 ± 0.01 <sup>b</sup>	0.43 ± 0.01 <sup>c</sup>
	Peel	0.46 ± 0.02 <sup>a</sup>	0.71 ± 0.04 <sup>a</sup>	0.98 ± 0.21 <sup>b</sup>
	Seed	13.14 ± 0.48 <sup>c</sup>	7.05 ± 0.59 <sup>a</sup>	9.94 ± 1.05 <sup>b</sup>
Methionine	Flesh	ND	ND	0.11 ± 0.00
	Peel	0.05 ± 0.01 <sup>a</sup>	0.18 ± 0.06 <sup>a</sup>	0.25 ± 0.10 <sup>b</sup>
	Seed	4.20 ± 0.37 <sup>ns</sup>	4.86 ± 0.37	4.96 ± 0.51
Phenylalanine	Flesh	0.07 ± 0.01 <sup>a</sup>	0.13 ± 0.02 <sup>a</sup>	0.49 ± 0.09 <sup>b</sup>
	Peel	0.41 ± 0.01 <sup>a</sup>	0.64 ± 0.01 <sup>b</sup>	0.94 ± 0.11 <sup>c</sup>
	Seed	15.52 ± 0.53 <sup>c</sup>	10.99 ± 0.91 <sup>a</sup>	13.14 ± 1.23 <sup>b</sup>
Proline	Flesh	0.07 ± 0.01 <sup>a</sup>	0.12 ± 0.02 <sup>b</sup>	0.44 ± 0.03 <sup>c</sup>
	Peel	0.36 ± 0.01 <sup>a</sup>	1.10 ± 0.16 <sup>b</sup>	1.21 ± 0.16 <sup>b</sup>
	Seed	11.98 ± 0.37 <sup>ab</sup>	12.98 ± 0.76 <sup>b</sup>	10.05 ± 1.68 <sup>a</sup>
Serine	Flesh	0.06 ± 0.01 <sup>a</sup>	0.12 ± 0.01 <sup>b</sup>	0.36 ± 0.01 <sup>c</sup>
	Peel	0.45 ± 0.03 <sup>a</sup>	0.56 ± 0.04 <sup>a</sup>	0.80 ± 0.14 <sup>b</sup>
	Seed	14.99 ± 0.21 <sup>c</sup>	9.58 ± 0.64 <sup>a</sup>	12.28 ± 0.82 <sup>b</sup>
Threonine	Flesh	0.03 ± 0.00 <sup>a</sup>	0.06 ± 0.00 <sup>b</sup>	0.28 ± 0.01 <sup>c</sup>
	Peel	0.26 ± 0.02 <sup>a</sup>	0.34 ± 0.04 <sup>a</sup>	0.64 ± 0.11 <sup>b</sup>
	Seed	7.56 ± 0.07 <sup>b</sup>	4.68 ± 0.46 <sup>a</sup>	6.86 ± 0.21 <sup>b</sup>
Tyrosine	Flesh	0.06 ± 0.01 <sup>a</sup>	0.08 ± 0.01 <sup>b</sup>	0.26 ± 0.04 <sup>c</sup>
	Peel	0.27 ± 0.06 <sup>a</sup>	0.26 ± 0.01 <sup>a</sup>	0.46 ± 0.10 <sup>b</sup>
	Seed	8.18 ± 0.01 <sup>b</sup>	4.74 ± 0.10 <sup>a</sup>	4.85 ± 1.21 <sup>a</sup>
Valine	Flesh	0.11 ± 0.02 <sup>a</sup>	0.18 ± 0.02 <sup>b</sup>	0.73 ± 0.05 <sup>c</sup>
	Peel	0.58 ± 0.01 <sup>a</sup>	0.71 ± 0.00 <sup>a</sup>	1.29 ± 0.17 <sup>b</sup>
	Seed	17.43 ± 0.69 <sup>c</sup>	10.79 ± 0.80 <sup>a</sup>	15.25 ± 0.78 <sup>b</sup>

<sup>1)</sup> Values are mean ± SD. Different superscript letters within a row indicate significant differences by Duncan's multiple range test ( $P < 0.05$ ).<sup>2)</sup> ND, not detected**Table 3.** Fatty acid concentrations (% fat) in pumpkin seeds (*Cucurbitaceae*) by species<sup>1)</sup>

Fatty acids	Species		
	<i>C. pepo</i>	<i>C. moschata</i>	<i>C. maxima</i>
Myristic acid (14:00)	0.23 ± 0.06	ND	0.16 ± 0.01
Palmitic acid (16:00)	12.97 ± 0.72 <sup>b</sup>	12.78 ± 0.11 <sup>b</sup>	10.84 ± 0.12 <sup>a</sup>
Heptadecanoic acid (17:00)	ND	ND	0.18 ± 0.01
Stearic acid (18:00)	4.67 ± 0.15 <sup>a</sup>	7.33 ± 0.20 <sup>c</sup>	5.84 ± 0.03 <sup>b</sup>
Oleic acid (18:1)	32.40 ± 0.56 <sup>c</sup>	31.34 ± 0.12 <sup>b</sup>	14.83 ± 0.05 <sup>a</sup>
Linoleic acid (18:2)	36.40 ± 0.82 <sup>a</sup>	35.72 ± 0.25 <sup>a</sup>	56.60 ± 0.29 <sup>b</sup>
Arachidic acid (20:00)	0.39 ± 0.06	ND	0.36 ± 0.02
Eicosenoic acid (20:1n-9)	ND	ND	0.07 ± 0.00
α-Linolenic acid (18:3n-3)	ND	ND	0.24 ± 0.01
Behenic acid (22:00)	0.37 ± 0.06	ND	0.09 ± 0.01
SFA	18.62 ± 0.64 <sup>b</sup>	20.11 ± 0.11 <sup>c</sup>	17.47 ± 0.13 <sup>a</sup>
MUFA	32.40 ± 1.66 <sup>c</sup>	31.34 ± 0.12 <sup>b</sup>	14.90 ± 0.04 <sup>a</sup>
PUFA	36.40 ± 0.82 <sup>a</sup>	35.72 ± 0.25 <sup>a</sup>	56.84 ± 0.29 <sup>b</sup>

<sup>1)</sup> Results are expressed as a % of the total fatty acid fraction. Values are mean ± SD. Different superscript letters within a row indicate significant differences by Duncan's multiple range test ( $P < 0.05$ ).

ND, not detected; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid

**Table 4.** Tocopherol (mg/kg raw weight) and carotenoid concentrations (mg/kg raw weight) in pumpkins (*Cucurbitaceae*) by species and by part<sup>1)</sup>

Tocopherols and carotenoids	Part	Species		
		<i>C. pepo</i>	<i>C. moschata</i>	<i>C. maxima</i>
α-Tocopherol	Flesh	1.40 ± 0.01	1.54 ± 0.99	2.31 ± 0.03
	Peel	4.49 ± 0.72 <sup>a</sup>	6.17 ± 2.19 <sup>ab</sup>	9.62 ± 0.79 <sup>b</sup>
	Seed	21.33 ± 3.65	25.74 ± 0.73	20.79 ± 1.33
γ-Tocopherol	Flesh	ND	0.52 ± 0.01	ND
	Peel	0.66 ± 0.09	ND	3.55 ± 0.17
	Seed	61.65 ± 17.66 <sup>b</sup>	66.85 ± 4.90 <sup>b</sup>	28.70 ± 2.13 <sup>a</sup>
β-Carotene	Flesh	1.48 ± 0.05	5.70 ± 0.39	17.04 ± 12.18
	Peel	39.48 ± 0.24 <sup>a</sup>	68.30 ± 2.02 <sup>ab</sup>	123.19 ± 30.61 <sup>b</sup>
	Seed	17.46 ± 18.29 <sup>ab</sup>	7.15 ± 1.50 <sup>a</sup>	31.40 ± 3.02 <sup>b</sup>
β-Cryptoxanthin	Flesh	ND	ND	0.65 ± 0.02
	Peel	0.15 ± 0.02 <sup>a</sup>	0.13 ± 0.03 <sup>a</sup>	6.57 ± 1.87 <sup>b</sup>
	Seed	0.16 ± 0.16	ND	0.21 ± 0.06

<sup>1)</sup> Values are mean ± SD. Different superscript letters within a row indicate significant differences by Duncan's multiple range test ( $P < 0.05$ ). ND, not detected

36.40 ± 0.82% fat, *C. moschata*: 35.72 ± 0.25% fat), but *C. maxima* seeds contained more linoleic acid (56.60 ± 0.29% fat) than oleic acid (14.83 ± 0.05% fat). *C. maxima* had 3-fold higher PUFA than MUFA. The PUFA content in *C. maxima* was significantly higher than *C. pepo* and *C. moschata* ( $P < 0.05$ ).

#### Tocopherol and carotenoid analysis

The tocopherol and carotenoid concentrations of the pumpkins are presented in Table 4. *C. maxima* had the highest α-tocopherol content in the peel, but the 3 species did not differ significantly. The α-tocopherol contents in the seeds of *C. pepo*, *C. moschata*, and *C. maxima* were 21.33 ± 3.65, 25.74 ± 0.73, and 20.73 ± 1.33 mg/kg raw weight, respectively. In the flesh, only *C. moschata*

**Table 5.**  $\beta$ -Sitosterol concentrations (mg/kg raw weight) in pumpkin seeds (*Cucurbitaceae*) by species<sup>1)</sup>

	Species		
	<i>C. pepo</i>	<i>C. moschata</i>	<i>C. maxima</i>
$\beta$ -sitosterol	383.89 $\pm$ 48.15 <sup>b</sup>	277.58 $\pm$ 23.48 <sup>a</sup>	235.16 $\pm$ 1.44 <sup>a</sup>

<sup>1)</sup> Values are mean  $\pm$  SD. Different superscript letters within a row indicate significant differences by Duncan's multiple range test ( $P < 0.05$ ).

contained  $\gamma$ -tocopherol. *C. pepo* and *C. maxima* peels contained  $\gamma$ -tocopherol. The  $\gamma$ -tocopherol contents of the seeds in *C. pepo* (61.65  $\pm$  17.66 mg/kg raw weight) and *C. moschata* (66.85  $\pm$  4.90 mg/kg raw weight) were higher than *C. maxima* seeds (28.70  $\pm$  2.13 mg/kg raw weight), ( $P < 0.05$ ). The peels in all 3 species had more  $\beta$ -carotene than the other parts. The  $\beta$ -carotene concentration in seeds was the highest in *C. maxima* (31.40  $\pm$  3.02 mg/kg raw weight).  $\beta$ -Cryptoxanthin was detected in the flesh of only *C. maxima*, in the peels of all 3 species, and in the seeds of *C. pepo* and *C. maxima*.

#### $\beta$ -Sitosterol

The  $\beta$ -sitosterol contents and presented in Table 5. *C. pepo* seeds had significantly more  $\beta$ -sitosterol (383.89  $\pm$  48.15 mg/kg raw weight), ( $P < 0.05$ ) than *C. moschata* and *C. maxima* (277.58  $\pm$  23.48 and 235.16  $\pm$  1.44 mg/kg raw weight, respectively).

## Discussion

The general chemical compositions and select bioactive components, including tocopherols, carotenoids, and  $\beta$ -sitosterol, were analyzed in 3 pumpkin species (*C. pepo*, *C. moschata*, and *C. maxima*) grown in Korea, and also in 3 parts (peel, flesh, and seed) of the pumpkin.

*C. maxima* had significantly more carbohydrates in the flesh than *C. pepo* and *C. moschata*. This high carbohydrate concentration may contribute to the sweet taste of *C. maxima*. Because of its sweet taste, *C. maxima* is called "Danhobak" in Korean, "Dan" meaning "sweet" and "hobak" meaning "pumpkin." The *C. maxima* flesh and peel had significantly more protein than *C. pepo* or *C. moschata*. *C. pepo* seeds had significantly more protein than *C. maxima* seeds ( $P < 0.05$ ). We found 20-25% more protein in the *C. pepo* seeds than reported in other studies [25-27], but 37-44% less protein than reported by Idouraine *et al.* [28]. We found 43.99-52.43% fat in the seeds, which is higher than the 24.2-45.1% reported for four *Cucurbita* species (*C. moschata*, *C. maxima*, *C. pepo*, and *C. argyrosperma*) grown in a common garden of Missouri, USA [15] and the 22-35% reported in African *C. pepo* [29]. *C. pepo* had the most moisture in all parts, and *C. maxima* had the lowest. The moisture contents in the current study were similar to previous reports for *C. maxima* (87.6%) and *C. moschata* (92.3%) [30].

The flesh and peel of *C. maxima* had more amino acids than the other two species. *C. pepo* seeds generally had more amino

acids than *C. moschata* and *C. maxima*. Pumpkin seeds contained all 9 essential amino acids. The histidine, leucine, and valine contents were higher than the other essential amino acids. The most significant difference in amino acid levels was for arginine. *C. pepo* seeds had over 6 times more arginine than *C. moschata* or *C. maxima*. The arginine content (63.99  $\pm$  0.88 mg/kg raw weight, 18.81%) of *C. pepo* seeds was similar to a previous report of 14-18% [28]. The amino acid profile of *C. moschata* in the current study was consistent with a previous study analyzing *C. moschata* cultivated in Korea [31].

In the current study, fatty acids were not analyzed in the flesh and peel because the fatty acid content in these parts was expected to be below the level of detection (0.1 g per 100 g edible pumpkin, data from the USDA nutrient database) [32]. Applequist *et al.* [15] detected stearic, palmitic, oleic, and linoleic acids in *C. pepo* seeds, while we detected 7 fatty acids in *C. pepo*, 4 fatty acids in *C. moschata*, and 10 fatty acids in *C. maxima*. The major fatty acids were palmitic (C16:0), stearic (C18:0), oleic (C18:1), and linoleic (C18:2) acids. Our results parallel several previous studies reporting that palmitic, stearic, and linoleic acid were the major fatty acids in pumpkin seeds [3,33]. *C. maxima* seeds contained 3-fold more PUFA than MUFA, which was significantly higher than *C. pepo* and *C. moschata* ( $P < 0.05$ ). The linoleic acid concentration in *C. moschata* seeds was higher than *C. pepo* in other studies [15,34]. In our study, the linoleic acid concentration in *C. maxima* was higher than other species ( $P < 0.05$ ).

Tocopherols and carotenoids have been suggested to be fat-soluble antioxidants. Antioxidants play an important role in decreasing DNA damage, diminishing lipid peroxidation, maintaining immune function, and inhibiting malignant transformation or proliferation *in vitro*, which are thought to prevent some diseases [35]. *C. maxima* had more  $\alpha$ -tocopherol in the flesh and peel than the other species; however, the differences were not significant ( $P > 0.05$ ).  $\gamma$ -Tocopherol was only present in *C. moschata* flesh, *C. pepo* and *C. maxima* peels, and the seeds of all 3 species. The  $\alpha$ -tocopherol content (2.31 mg/kg) in *C. maxima* flesh was much lower than reported in the USDA nutrient database (1.06 mg/100 g edible pumpkin) [32]. The  $\alpha$ - and  $\gamma$ -tocopherol levels in pumpkin seeds in this study were lower than reported for 12 pumpkin seed cultivars from the USA [2]. Stevenson *et al.* [2] reported  $\alpha$ -tocopherol and  $\gamma$ -tocopherol contents between 27.1-75.1 mg/kg and 74.9-492.8 mg/kg, respectively.  $\gamma$ -Tocopherol contents in *C. pepo* and *C. moschata* seeds were typically 2.5-3.0 times higher than  $\alpha$ -tocopherol.  $\alpha$ -Tocopherol has the greatest bioavailability, however,  $\gamma$ -tocopherol may have higher antioxidant activities [36,37]. Whang *et al.* [38] reported that  $\beta$ -carotene contents in the flesh and peel of *C. moschata* cultivated in Korea were similar. In this study, the  $\beta$ -carotene contents in the peels of 3 species were 5-15 folds higher than in the flesh.

Each pumpkin part in this study contained a significant amount of antioxidants, tocopherols, and carotenoids. Therefore, pumpkin

potentially has antioxidant activity, which might be important for pre-diabetics, diabetics, and patients with vascular injury [39]. Administering pumpkin extract significantly increased superoxide dismutase and glutathione peroxidase activities in mouse liver [40]. Diets high in pumpkin seeds have been associated with lower risks of gastric, breast, lung, and colorectal cancers [41]. The carotenoids in pumpkin flesh might prevent prostate cancer [42]. In addition to fat-soluble antioxidants (tocopherols and carotenoids), *C. maxima* had 16 mg vitamin C per 100 g raw pumpkin [10]. Vitamin C is a strong water-soluble antioxidant that protects cells and cellular components from free radicals by donating electrons, and regenerating other antioxidants, such as vitamin E (tocopherols) [43]. Therefore, high pumpkin intake has various benefits to improve overall health. Currently, pumpkins are consumed as vegetables and medicines in many countries, such as China, Argentina, India, Mexico, Brazil, the US, and Korea. It is commonly used to prevent diabetes and eliminate intestinal parasites [44]. In Korea, pumpkins have been used traditionally to relieve edema during pregnancy and after delivery. Among the 3 species in this study, extracts of *C. maxima* and *C. moschata* flesh are frequently used as a medicine in Korea [45]. Although the peels are usually discarded in Korea, they contain much more tocopherols and carotenoids than the flesh, thus they may have a domestic use as medicine.

$\beta$ -Sitosterol is a phytosterol, which are integral components of plant cell membranes, and are abundant in vegetable oils, nuts, seeds, and grains [46]. Phytosterols can lower both total serum cholesterol and LDL-cholesterol in humans by inhibiting the absorption of dietary cholesterol [47], and can prevent cancer [48]. Recently, plant sterols have been proposed to have other positive health effects [49].  $\beta$ -Sitosterol especially is considered a treatment for benign prostatic hyperplasia [8]. *C. pepo* seeds had significantly more  $\beta$ -sitosterol ( $383.89 \pm 48.15$  mg/kg raw weight), ( $P < 0.05$ ) than *C. moschata* and *C. maxima* ( $277.58 \pm 23.48$  and  $235.16 \pm 1.44$  mg/kg raw weight, respectively). The  $\beta$ -sitosterol content in *C. pepo* in this study was similar to barley (381 mg/kg) and maize (341 mg/kg) [5]. Ryan *et al.* [5] reported that the  $\beta$ -sitosterol content in pumpkin seeds was 249 mg/kg, which is similar to *C. moschata* and *C. maxima* in our study. The pumpkin seeds in this study (cultivated in Korea) had more  $\beta$ -sitosterol than pumpkin seed oils cultivated in the USA [1]. The high  $\beta$ -sitosterol contents in this study may result from the cultivars, growing seasons, and planting locations, which maximize the phytosterol concentrations in plants [1].  $\beta$ -Sitosterol might have broad biological effects including lowering cholesterol, estrogenic activity, and anticarcinogenic activity [48,49]. Therefore, pumpkin seeds highly containing  $\beta$ -sitosterol would help maintain human health.

In summary, the amino acid contents were higher in the seeds than the flesh or peel. Amino acid contents in *C. pepo* seeds were higher than *C. moschata* and *C. maxima*. The major fatty acids were palmitic, stearic, oleic, and linoleic acid. The  $\alpha$ -tocopherol concentration was highest in *C. pepo* peel, but the

3 species did not differ significantly.  $\gamma$ -Tocopherol was detected in the seeds of all species. There was no significant difference in  $\beta$ -carotene contents of the flesh and peel. The  $\beta$ -carotene content in seeds was highest in *C. maxima*. *C. pepo* seeds had significantly more  $\beta$ -sitosterol than *C. moschata* and *C. maxima*. This study should help updating nutrient compositions in the Korean food composition database, as well as estimate more accurate dietary intake and nutrient adequacies from food consumption surveys in Korea. Further research on the nutrient composition of pumpkins is needed, including analyses of various pumpkins grown in different years and different areas of Korea.

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