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A comparative study of nutrient compositions between HongJams prepared from 5 silkworm varieties making white cocoons

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Abstract

White-Jade silkworm (previously also known as Baegokjam) variety is the most popular silkworm variety that produces white cocoons. In 2021, the market share of White-Jade variety in Korea is very high, accounting for 88% of the silkworm production. Daebaekjam, Dodam-silkworm, Kumkangjam, and Kumokjam varieties, which have recently been established, make white cocoons like White-Jade. In this study, we found that 5 types of HongJams produced from 5 varieties of silkworms producing white cocoons did not show any severe difference in proximate analysis. The amounts of crude proteins, the most abundant nutrient, were between 71.05 ~ 73.38%, and those of crude lipids were 13.89 ~ 14.69% in 5 types of HongJams. In addition, there was no difference between White-Jade HongJam (WJ) and Daebaekjam HongJam in amino acid compositions. The amount of unsaturated fatty acid was significantly higher in WJ than in the other four types of HongJams, but the omega-6 fatty acids/omega-3 fatty acids ratio was higher in the four types of HongJams. Most of the minerals were higher in four types of HongJams than in WJ, and three heavy metal were not detected in all 5 types of HongJams. Phytochemicals were also most abundant in WJ, but the difference in the amounts were not severe. And pepsin digestibility was the highest for Kumokjam HongJam and the lowest for Dodam-silkworm HongJam, but the difference was not severe. The nutritional component analysis results of this study suggested that four new varieties can be used for producing HongJams, and Daebaekjam can replace White-Jade the most as the protein source.

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Introduction

Humans have been breeding silkworms for more than 8000 years to produce silk fabrics and high-quality protein and lipid nutrients from cocoons and pupae, respectively (Kim and Koh, 2022; Park *et al*., 2022). In addition, the health-promoting

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effects of various products of silkworms are mentioned in the diverse traditional and ancient oriental medicine literature. The Rural Development Administration (RDA) in Korea has been conducting scientific research on various health-promoting effects of silkworm products mentioned in the traditional oriental medicine literature to maintain the agricultural and industrial basis of sericulture (Kim and Koh, 2022; Koh, 2020; Nguyen *et al*., 2020a; Park *et al*., 2022). From the 1990s, preclinical and clinical trials demonstrated the hypoglycemic effect of freeze-dried silkworms on the 3rd-day of 5th-instar larvae (Ryu *et al*., 2002b; Ryu *et al*., 2012} and the improvement of sexual function-promoting effects of male pupa alcohol extracts (Ryu *et al*., 2002a; Ryu *et al*., 2010). In addition, it has been demonstrated that silkworm Dongchunghacho (*Paecilomyces japonica*) had hypoglycemic and immune-promoting effects (Ji *et al*., 2012; Lee *et al*., 2017). Recently, RDA has developed a technology to manufacture HongJam which has varieties of health-promoting effects by processing mature silkworms contained hypertrophied silk glands (Ji *et al*., 2015). The results of the studies using diverse HongJams prepared from various silkworm varieties with different cocoon colors revealed that the best indications for HongJams were different depending on the cocoon color of silkworms used (Cho *et al*., 2016; Kim *et al*., 2019; Mai *et al*., 2022; Nguyen *et al*., 2020b). For examples, HongJam made with White-Jade (previously also known as Baegokjam) mature silkworms (WJ) which makes white cocoons (Lee *et al*., 1984) has liver function improvement and gastro-intestinal protection effects (Cho *et al*., 2016; Yun *et al*., 2017). In addition, Golden-Silk HongJam (GS) (Kang *et al*., 2007) which made with mature silkworms producing yellow-colored cocoons has been shown to inhibit the onset of Parkinson's disease and Alzheimer's disease and promote diverse memory functions (Choi *et al*., 2017; Mai *et al*., 2022; Nguyen *et al*, 2016; Nguyen *et al*., 2020b; Nguyen *et al*., 2021)

The current representative silk moth variety in Korea is White-Jade silkworms which make white cocoons and were established in 1984 (Lee *et al*., 1984). White-Jade silkworms are now most bred and used for producing the freeze-dried 5th-instar and 3rd-day larval powders. White-Jade mature silkworm larvae are used for producing HongJam. Kumokjam which makes white cocoons were established 1994 (Kim *et al*., 2015) and now 2nd most bred variety. Recently, additional silkworm varieties making white cocoons such as Kumkangjam (2013), Dodam-silkworm (2014), and Daebackjam (2017) were established. Since recently established silkworm varieties have more mature larval weight than White-jade mature silkworms, using new silkworm variety with heavier larval weights will be great benefit for sericulture farms and industries. Thus, we investigate nutrient composition differences among the five different types of HongJams produced from five mature silkworm varieties making white cocoons.

Materials and Methods

HongJam production

White-Jade silkworm is F1 of the cross of pure breed (PB) Jam 123 x PB Jam 124. Daebaekjam is F1 of the cross of PB Jam 157 x PB Jam 162. Dodam-silkworm is F1 of the cross of PB Jam 161 x PB Jam 162. Kumkangjam is F1 of the cross of PB Jam 259 x PB Jam 160. Kumokjam is F1 of the cross of PB Jam 125 x PB Jam 140. Larvae of 5 silkworm varieties were cultured using mulberry leaves in the National Institute of Agricultural Sciences (NIAS) by mature silkworms which were the 7 to 9-day of 5th instar larvae. After the selected mature silkworms were washed with tap water, they were steamed for 120 minutes using a steamer without pressure. The steamed silkworms, those are known as HongJam, were immediately frozen and then freeze-dried at -50 °C for 48 hours before pulverizing with a natural stone roller mill. The powders of White-Jade HongJam (WJ), Deabackjam HongJam (DBJ), Dodam-silkworm HongJam (DDS), Kumkangjam HongJam (KKJ), and Kumokjam HongJam (KOJ) were used to analyze nutrients.

The weight of mature silkworms of 5 varieties raised during the fall season 2022 were measured. Total 300 larvae for each variety were measured. The mean weight of White-Jade mature silkworm was 395.3 ± 3.48 while those of Daebackjam, Dodam-silkworm, Kumokjam, and Kumkangjam were 403 ± 4.09, 423.7 ± 3.53, 374.7 ± 2.03, and 419.0 ± 2.08, respectively. Dodam-silkworm was the heaviest and Kumokjam was the lightest ($F_{(4, 14)} = 38.791$, $P < 0.001$).

Proximate analysis

A proximate analysis to measure the amount of moisture, crude protein, crude lipid, crude fiber, and crude ash present in 5 kinds of HongJam powders was carried out using the previously published protocols registered in the Korea food code (Ji *et al*., 2016a, 2016b, 2019). Briefly, moisture content was measured by drying at 105°C by atmospheric drying method and crude protein was analyzed using an automatic protein analyzer (Kjeltec 2400 AUT, Foss Tecator, Hilleroed, Denmark) by the semimicro-Kjeldhl method. Crude lipid was extracted with diethyl ether and quantified by using a Soxhlet extractor (Soxtec System HT 1043 extraction unit, Foss Tecator). Crude ash was measured by direct ashing at 600°C. Crude fiber was measured by decomposition of samples in 1.25% H_2SO_4 and NaOH.

Amino acid composition analysis

The compositions of 18 amino acids in 5 types of HongJam powders were analyzed. After adding 6N HCL solution to a certain amount of sample, injecting nitrogen gas, hydrolyzing at 110°C for 24 hours, removing HCl using a rotary evaporator, washing with distilled water three times, and then concentrating under reduced pressure. The concentrate was dissolved in distilled water and analyzed according to the manufacturer's instructions using an automatic amino acid analyzer (Hitachi L-8900A, Hitachi, Tokyo, Japan).

Fatty acid composition analysis

The compositions of fatty acids in 5 types of HongJam powders were analyzed by the Folch method (Folch *et al*., 1957; Ji *et al*, 2016a, 2016b, 2019). The lipids extracted with the extraction solution (chloroform:MeOH = 2:1) were dehydrated by anhydrous Na_2SO_4, concentrated. Sequentially adding tricosanic acid and 0.5 N NaOH, samples were heated at 100°C for 20 min. After cooling down at RT for 30 min, samples were mixed with BF3-methanol, and then heated for 20 min and cooled down at RT for 30 min. Heptane and Nacl (1:8) were added to separate supernatants which were analyzed by a gas chromatography (Agilent US/HP 6890, Agilent Technologies, Santa Clara, CA, USA).

Mineral composition analysis

Preliminarily incinerate an appropriate amount of the sample using a crusible, incinerate at 600°C for 2 hours or more, and then allow to cool. Add 10 ml of hydrochloric acid solution (1:1 diluted with dH_2O) to the sample, let it stand overnight to dissolve it, and then filter it with hot water using No.6 filter paper to adjust the amount of the sample solution. The sample solution is analyzed using an Inductively Coupled Plasma Optical Emission Spectrometer (GBC Scientific Equipment, Victoria, Australia).

Phytochemical analysis

Quantifying total polyphenol

To measure total polyphenol, the sample is mixed with 80% ethanol (V/V), stirred for 20 minutes using a shaker, and then ultrasonically extracted at 70°C for 1 hour, followed by centrifugation (20,000 rpm, 15 minutes) to take the supernatant. Take 1 ml of the standard solutions and test solutions into a test tube, add 8 ml distilled water and 1 mL Folin & Ciocalteu's Phenol reagent (1 N), stir thoroughly, and after 5 minutes add 1 mL Na_2CO_3 solution (15%) and 1 ml Na_2CO_3 solution (15%) and leave at room temperature (RT) for 2 hours. After that, the absorbance is measured at 760 nm. A calibration curve was prepared using the difference between the absorbance by the concentration of the standard material and the absorbance of the blank test for each concentration. The calibration curve was prepared using catechin as a standard material. The amount of total polyphenol was expressed in mg catechin equivalent per 100 g of sample weight.

Quantifying total flavonoid

The supernatant used for quantifying total polyphenol was used. To 0.5 mL of the control or test solutions, 2.0 mL of dH_2O and 0.15 mL $NaNO_2$ (5%) were added. After stirred for 6 min, 0.15 mL $AlCl_36H_2O$ solution (10%) was added, left for 5 min, and then 1 mL NaOH solution (1 M) was added. After stirring and standing at RT for 15 min, absorbance at 510 nm was measured and the reagent blank was used as a control solution. A calibration curve of the standard material was prepared using the difference between the absorbance by the various concentrations of the standard material and the absorbance of the reagent blank. A calibration curve was prepared using quercetin as the standard material The quantity of total flavonoid was expressed as mg quercetin equivalent per 100 g of sample weight.

Pepsin digestibility analysis

Add 150 ml of 0.2% pepsin in 0.1 N HCl solution to the defatted sample and digest at 45°C for 16 hours. The sample is filtered with No. 2 filter paper to analyze the crude protein, and the pepsin digestibility is calculated from the content of undigested and pepsin-digested crude protein.

Table 1. Proximate nutrient compositions of HongJams made from five silkworm varieties making white cocoons

Crude nutrient	Silkworm varieties				
	WJ (BOJ)	DBJ	DDS	KOJ	KGJ
	Mean ± SE (%)				
moisture	1.78 ± 0.034[a]	2.13 ± 0.043[b]	1.92 ± 0.061[ab]	1.67 ± 0.104[a]	1.88 ± 0.055[a]
protein	73.38 ± 0.139[a]	71.05 ± 0.719[b]	72.15 ± 0.188[a]	71.60 ± 0.119[b]	72.26 ± 0.08[b]
lipid	13.89 ± 0.548[a]	14.69 ± 0.275[a]	14.64 ± 0.028[a]	14.60 ± 0.011[a]	14.02 ± 0.247[a]
fiber	7.02 ± 0408[a]	7.59 ± 0.863[a]	7.50 ± 0.2383[a]	8.03 ± 0.133[a]	8.00 ± 0.353[a]
ash	3.93 ± 0.008[a]	4.53 ± 0.012[b]	3.79 ± 0.007[c]	4.1 ± 0.021[d]	3.83 ± 0.001[e]

Statistical differences between HongJams were determined by one-way ANOVA followed by Tukey's HSD post hoc analysis. Cells showing significant differences compared to WJ were shaded.
[a, b, c, d, e] Different letters indicate significant differences at $P < 0.05$.
The cells with significant differences compared to WJ were shaded.

Results and Discussion

No significant difference in the crude nutrients of HongJam prepared from 5 types of silkworms that make white cocoons.

Proximate analysis was performed as a preliminary study to find out whether there was a difference in nutritional composition among the HongJams made from 5 different silkworm varieties. As you can see clearly from the results summarized in Table 1, only DBJ had a more significant amount of moisture than WJ (Table 1, $F_{(4, 14)} = 7.291$, $P < 0.01$), but the difference was less than 0.5%. Generally, the recommended content of moisture in dried food for long-term storage is < 10% (Afolabi, 2014). Thus, it is predicted that the difference in moisture between 5 types of HongJams will not give significant differences while long-term storing of them.

The differences in the crude protein content of the 5 types of HongJams showed that three HongJams except for DDS had a lower content than that of WJ (Table 1, $F_{(4, 14)} = 6.35$, $P < 0.01$). However, the difference in protein content between the 5 types of HongJams is up to 1.8%, thus, it is not expected that there will be a significant difference in functionality between HongJams. In addition, there were no statistical differences in the contents of lipid (Table 1, $F_{(4, 14)} = 1.658$, $P > 0.05$) and fibers (Table 1, $F_{(4, 14)} = 0.776$, $P > 0.05$) between 5 types of HongJams. Compared to WJ, DBJ and KOJ had significantly more crude ash contents while DDS and KGJ had significantly less (Table 1, $F_{(4, 14)} = 564.69$, $P < 0.001$). However, the crude ash content differences between HongJams were 0.15 ~ 0.6%, which had no nutritional significance. The crude protein contents of 5 types of HJs were higher than HongJams produced with silkworms weaving different colored-cocoons (Ji *et al*., 2016a), suggesting that HongJams made from white-cocoon silkworms might be wonderful protein sources.

No significant difference in amino acid composition between 5 HongJams

Since protein is the most abundant nutrient, accounting for 71 to 73% of HongJam (Table 1), we conducted a study comparing the amino acid composition of 5 types of HongJams (Table 2). Compared to those of WJ, there were no statistically different amino acid contents in DBJ. The contents of CYS ($F_{(4, 14)} = 2.969$, $P > 0.05$), MET ($F_{(4, 14)} = 2.192$, $P > 0.05$) GLY ($F_{(4, 14)} = 2.364$, $P > 0.05$), ASP ($F_{(4, 14)} = 1.722$, $P > 0.01$), ALA ($F_{(4, 14)} = 1.722$, $P > 0.05$), VAL ($F_{(4, 14)} = 1.747$, $P > 0.05$), ILE ($F_{(4, 14)} = 2.649$, $P > 0.05$), TYR ($F_{(4, 14)} = 1.639$, $P > 0.05$), ARG ($F_{(4, 14)} = 2.954$, $P > 0.05$), PRO ($F_{(4, 14)} = 3.484$, $P > 0.05$), and TRP ($F_{(4, 14)} = 0.352$, $P > 0.05$) in 5 HongJams were not significantly different (Table 1). However, the content of ASP in DDS, KOJ, and KGJ were significantly less than that of WJ ($F_{(4, 14)} = 2.364$, $P > 0.05$). However, the contents of THR ($F_{(4, 14)} = 4.027$, $P < 0.01$), GLU ($F_{(4, 14)} = 8.643$, $P < 0.05$), LEU ($F_{(4, 14)} = 5.309$, $P < 0.05$), PHE ($F_{(4, 14)} = 7.837$, $P < 0.05$), LYS ($F_{(4, 14)} = 3.891$, $P < 0.05$), and HIS ($F_{(4, 14)} = 5.372$, $P < 0.05$) in DDS were significantly lower than those of WJ (Table 2, . In case of KOJ, the contents of ASP, THR, SER, and HIS were significantly less than those of WJ. And the contents of ASP, THR, GLU, PHE, and HIS in KGJ were significantly less than those of WJ. However, total amino

Table 2. Amino acid composition analysis results of five different HongJam powders

Amino acids	Silkworm varieties				
	WJ (BOJ)	DBJ	DDS	KOJ	KGJ
	Mean ± SE (%)				
Cysteine (CYS)	0.30 ± 0.007[a]	0.30 ± 0.011[a]	0.33 ± 0.012[a]	0.31 ± 0.005[a]	0.33 ± 0.009[a]
Methionine (MET)	0.69 ± 0.025[a]	0.71 ± 0.024[a]	0.72 ± 0.028[a]	0.70 ± 0.006[a]	0.77 ± 0.018[a]
Glycine (GLY)	13.30 ± 0.139[a]	12.84 ± 0.089[a]	13.33 ± 0.188[a]	12.76 ± 0.248[a]	12.96 ± 0.138[a]
Aspartic acid (ASP)	5.14 ± 0.044[a]	5.04 ± 0.04[a]	4.79 ± 0.056[a]	4.90 ± 0.118[a]	4.89 ± 0.41[a]
Threonine (THR)	2.27 ± 0.019[a]	2.23 ± 0.018[ab]	2.12 ± 0.047[b]	2.12 ± 0.05[b]	2.15 ± 0.014[b]
Serine (SER)	6.29 ± 0.130[a]	6.11 ± 0.139[a]	5.87 ± 0.163[a]	5.63 ± 0.161[b]	5.79 ± 0.055[a]
Glutamic acid (GLU)	4.71 ± 0.074[a]	4.58 ± 0.049[ab]	4.16 ± 0.09[b]	4.43 ± 0.099[ab]	4.32 ± 0.027[b]
Alanine (ALA)	10.60 ± 0.124[a]	10.28 ± 0.024[a]	10.53 ± 0.096[a]	10.23 ± 0.216[a]	10.34 ± 0.038[a]
Valine (VAL)	2.87 ± 0.034[a]	2.87 ± 0.047[a]	2.73 ± 0.04[a]	2.80 ± 0.055[a]	2.79 ± 0.049[a]
Isoleucine (ILE)	1.64 ± 0.005[a]	1.61 ± 0.023[a]	1.51 ± 0.027[a]	1.58 ± 0.039[a]	1.57 ± 0.039[a]
Leucine (LEU)	2.18 ± 0.019[a]	2.20 ± 0.005[a]	2.02 ± 0.019[b]	2.14 ±0.058[ab]	2.10 ± 0.031[ab]
Tyrosine (TYR)	4.73 ± 0.031[a]	4.48 ±0.076[a]	4.52 ± 0.08[a]	4.52 ± 0.108[a]	4.52 ± 0.070[a]
Phenylalanine (PHE)	2.19 ± 0.026[a]	2.22 ± 0.019[a]	2.02 ± 0.032[b]	2.19 ± 0.050[a]	2.04 ± 0.033[b]
Lysine (LYS)	2.78 ± 0.014[a]	2.70 ± 0.012[ab]	2.55 ± 0.031[b]	2.69 ± 0.071[ab]	2.65 ± 0.009[ab]
Histidine (HIS)	1.26 ± 0.015[ab]	1.38 ± 0.0134[a]	1.23 ± 0.021[b]	1.24 ± 0.045[b]	1.23 ± 0.024[b]
Arginine (ARG)	2.22 ± 0.005[a]	2.17 ± 0.027[a]	2.08 ± 0.023[a]	2.13 ± 0.059[a]	2.10 ± 0.027[a]
Proline (PRO)	1.65 ± 0.055[a]	1.64 ± 0.024[a]	1.53 ± 0.012[a]	1.52 ± 0.044[a]	1.63 ± 0.013[a]
Trypophan (TRP)	0.57 ± 0.011[a]	0.57 ± 0.020[a]	0.57 ± 0.010[a]	0.57 ± 0.019[a]	0.59 ± 0.014[a]
Total	65.38 ± 0.662[a]	63.94 ± 0.333[a]	62.58 ± 0.769[a]	62.44 ± 1.386[a]	62.79 ± 0.425[a]

Statistical differences between HongJams were determined by one-way ANOVA followed by Tukey's HSD *post hoc* analysis.
[a, b] Different letters indicate significant differences at $P < 0.05$.
The cells with significant differences compared to WJ were shaded.

acid contents in 5 HongJams were not significantly different ($F_{(4, 14)} = 2.370$, $P < 0.05$). In previous studies, we have compared the amino acid content differences between HongJams generated from silkworms weaving different colored-cocoons (Ji *et al*, 2016a;Ji *et al*., 2016b). Those studies have shown that WJ has higher protein and amino acid contents than other HongJams, such as Golden-silk (yellow cocoon), Red-Silk (red cocoon), or Pistachio-Silk (Light green cocoon). Thus, our finding that there were no significant differences in the amounts of crude proteins and the contents of amino acids between WJ and DBJ suggested that DBJ can be the best replacement variety for WJ which is used for providing proteins. Nevertheless, the other three silkworm varieties used in this study did not have significant differences from WJ and DBJ in terms of nutrient values.

The contents of unsaturated fatty acids in WJ were highest

Fatty acids which are energy sources and have antioxidant activities abundantly exist in 5 types of HongJam powders (Table 3). Compared to WJ, DBJ and DDS had significantly more three saturated fatty acids, while KOJ and KGJ had only significantly more Palmitic acid and Stearic acid (Myristic acid, $F_{(4, 14)} = 6.676$, $P < 0.01$; Palmitic acid, $F_{(4, 14)} = 1782,078$, $P < 0.001$; Stearic acid, $F_{(4, 14)} = 932.454$, $P < 0.001$). Among the unsaturated fatty acids, Palmitoleic acid ($F_{(4, 14)} = 226,979$, $P < 0.001$), Oleic acid ($F_{(4, 14)} = 433.864$, $P < 0.001$), and Linolenic acid ($F_{(4, 14)} = 407.183$, $P < 0.001$) were present in significantly lower amounts in the 4 types of HongJams compared to WJ. In contrast, Linoleic acid ($F_{(4, 14)} = 1190.835$, $P < 0.001$) in DBJ, DDS, KOJ

Table 3. Types and ratios of saturated and unsaturated fatty acids exiting in 5 different HongJam powders

Silkworm varieties		WJ (BOJ)	DBJ	DDS	KOJ	KGJ
Saturated fatty acid	Myristic acid	0.16 ± 0.002[a]	0.17 ± 0.002[b]	0.18 ± 0.002[b]	0.17 ± 0.004[a]	0.17 ± 0.004[a]
	Palmitic acid	22.66 ± 0.025[a]	25.26 ± 0.002[b]	24.74 ± 0.011[b]	24.79 ± 0.006[b]	23.79 ± 0.001[c]
	Stearic acid	7.17 ± 0.036[a]	9.68 ± 0.011[b]	9.32 ± 0.032[c]	8.25 ± 0.052[d]	8.06 ± 0.018[e]
Unsaturated fatty acid	Palmitoleic acid	0.82 ± 0.003[a]	0.73 ± 0.002[b]	0.8 ± 0.001[c]	0.82 ± 0.005[a]	0.85 ± 0.003[d]
	Oleic acid	24.77 ± 0.028[a]	25.15 ± 0.002[b]	25.18 ± 0.044[b]	26.55 ± 0.084[c]	26.91± 0.026[d]
	Linoleic acid	7.13 ± 0.005[a]	6.48 ± 0.009[b]	6.64 ± 0.007[c]	6.50 ± 0.011[b]	6.77 ± 0.006[a]
	γ-Linoleic acid	0.1 ± 0.001[a]	0.11 ± 0.001[b]	0.10 ± 0.001[a]	0.10 ± 0.001[a]	0.13 ± 0.001[c]
	Linolenic acid	37.13 ± 0.088[a]	32.36± 0.022[b]	32.98 ± 0.087[c]	32.75 ± 0.169[c]	33.25 ± 0.046[d]
	Eicosenoic acid	0.07 ± 0.007[a]	0.06 ± 0.004[a]	0.06 ± 0.004[a]	0.06 ± 0.004[a]	0.06 ± 0.006[a]
Total Saturated fatty acid		29.99 ± 0.060[a]	35.11 ± 0.010[b]	34.24 ± 0.046[c]	33.21 ± 0.097[d]	32.02 ± 0.026[a]
Total Unsaturated fatty acid		70.01 ± 0.060[a]	64.89 ± 0.010[b]	65.76 ± 0.046[b]	66.79 ± 0.097[dc]	67.98 ± 0.002[c]
Mono-unsaturated		25.65 ± 0.031[a]	25.94 ± 0.044[b]	26.04 ± 0.048[b]	27.43 ± 0.079[c]	27.83 ± 0.029[d]
Poly-unsaturated		44.36 ± 0.089[a]	38.95 ± 0.014[b]	39.72 ± 0.093[c]	39.35 ± 0.175[c]	40.15 ± 0.051[d]
n-6/n-3 ratio		0.194 ± 0.0005[a]	0.204 ± 0.0004[bc]	0.205 ± 0.0004[b]	0.202 ± 0.0008[c]	0.207 ± 0.0002[bd]

Statistical differences were determined by one-way ANOVA followed by HSD post hoc analysis.
[a, b, c, d, e] Different letters indicate significant differences at $P < 0.05$.
The cells with significant differences compared to WJ were shaded.

were significantly lower and γ-Linoleic acid ($F_{(4, 14)} = 249.523$, $P < 0.001$) were significantly higher in DBJ and KGJ than those of WJ. There was no significant different in the content of Eicosenoic acid in 5 types of HongJams ($F_{(4, 14)} = 0.253$, $P > 0.05$). Total saturated acid in DBJ, DDS, and KOJ was significantly higher than in WJ, but WJ had significantly more total unsaturated fatty acid than the other four HongJams ($F_{(4, 14)} = 1254.519$, $P < 0.001$). However, as mentioned above, there is no significant difference in the contents of crude lipids in 5 types of HongJams, so they are predicted to have similar health improvement effects. Compared with previous results of HongJam prepared from silkworms producing different colored-cocoons ({Ji *et al*, 2016a; Ji *et al*., 2016b), the saturated/unsaturated fatty acid ratios of DBJ, DDS, KOJ and KGJ were similar to those of Golden-silk and Red-silk HongJam. In addition, when the amounts of omega-6 (n-6) fatty acids, which have been controversially associated with the development of atherosclerosis and cardiovascular diseases (DiNicolantonio and O'Keefe, 2018 ; Simopoulos, 2002; Walz *et al*., 2016), were divided by the amounts of omega-3 (n-3) in 5 types of HongJams, the n-6/n-3 fatty acid ratio of WJ was the lowest, 0.194. The n-6/n-3 fatty acid ratios of the other 4 types of HongJams were also between 0.202 to 0.207, which was lower than those of HongJam prepared from silkworms with other colors, indicating that all 5 types of HongJams have good fatty acid composition. Therefore, we can suggest that a large amount of fatty acid in HongJam could be good for health promotion.

DBJ shows the highest mineral content

It has been previously reported that HongJam contains a large amount of minerals (Ji *et al*., 2016a, 2016b, 2019). When the minerals present in 5 types of HongJams were compared, 4 types of HongJams had higher amounts of Ca ($F_{(4, 14)} = 10775.81$, $P < 0.001$), Fe ($F_{(4, 14)} = 49.557$, $P < 0.001$), K ($F_{(4, 14)} = 50.856$, $P < 0.001$), Mg ($F_{(4, 14)} = 2355.263$, $P < 0.001$), Mn ($F_{(4, 14)} = 515.223$, $P < 0.001$), and *P* ($F_{(4, 14)} = 191.702$, $P < 0.001$) than WJ (Table 4). The amounts of Cu ($F_{(4, 14)} = 142.218$, $P < 0.001$) and Zn ($F_{(4, 14)} = 253.236$, $P < 0.001$) in DDS was not different from those of WJ, but DBJ, KOJ, and KGJ had significantly less than those of WJ. The three regulated heavy metals such as Pb, Cd and As were not detected in all 5 types of HongJams. These mineral and heavy metal analysis results suggested that 5 types of HongJams are safe from heavy metal contaminations and contain key minerals for maintaining normal physiological functions of organs and tissues.

One of the possible health-benefit which humans can obtain

Table 4. The amounts of minerals in 5 different HongJam powders

Minerals (mg/100g)	Silkworm varieties				
	WJ (BOJ)	DBJ	DDS	KOJ	KGJ
Calcium (Ca)	205.4 ± 0.48[a]	295.2 ± 0.21[b]	208.2 ± 0.23[c]	241.8 ± 0.27[d]	229.3 ± 0.46[e]
Copper (Cu)	0.11 ± 0.005[a]	0.13 ± 0.009[b]	0.07 ± 0.007[a]	0.31 ± 0.008[c]	0.36 ± 0.019[d]
Iron (Fe)	1.23 ± 0.017[a]	1.48 ± 0.027[b]	1.36 ± 0.008[c]	1.59 ± 0.008[c]	1.52 ± 0.030[bc]
Potassium (K)	894.0 ± 6.56[a]	1052.5 ± 6.21[b]	921.0 ± 8.42[c]	1035.6 ± 5.63[b]	1016.4 ± 17.86[b]
Magnesium (Mg)	205.6 ± 0.19[a]	220.4 ± 0.06[b]	209.1 ± 0.15[c]	229.8 ± 0.12[d]	226.4 ± 0.39[e]
Manganese (Mn)	1.07 ± 0.011[a]	1.51 ± 0.009[b]	1.19 ± 0.009[c]	1.44 ± 0.007[d]	1.15 ± 0.004[e]
Sodium (Na)	22.48 ± 0.772[a]	18.68 ± 0.888[ab]	17.73 ± 0.953[bc]	33.19 ± 1.297[d]	14.95 ± 0.985[c]
Phosphorus (P)	699.3 ± 1.28[a]	743.1 ± 0.82[b]	718.9 ± 1.22[c]	715.9 ± 1.41[c]	707.9 ± 1.13[d]
Zinc (Zn)	6.14 ± 0.017[a]	5.34 ± 0.009[b]	6.12 ± 0.013[a]	6.22± 0.023[c]	5.90 ± 0.039[d]
Lead (Pb)	-	-	-	-	-
Cadmium (Cd)	-	-	-	-	-
Arsenic (As)	-	-	-	-	-

Statistical differences were determined by one-way ANOVA followed by HSD post hoc analysis.
[a, b, c, d, e] Different letters indicate significant differences at $P < 0.05$.
The cells with significant differences compared to WJ were shaded.

from HongJam is reducing blood pressure. Hypertension is a metabolic disease caused by various causes, and one of the main causes is excessive intake of Na (Grillo *et al*., 2019). One of the ways to lower hypertension is to eat foods with a high K/Na ratio (Burnier, 2018). Interestingly, the 5 types of HongJams had a large amount of K and a small amount of Na (Table 1). The ratio of K/Na in WJ was 39.77. The K/Na ratios of KGS, DBJ, DDS, and KOJ were 67.98, 56.34, 51.95, and 31.20, respectively. Only K/Na ratio of KOJ was lower than that of WJ. These results suggested there were no significant differences in mineral contents between the 5 types of HongJams, but DBJ had the most abundant minerals with ratios good for health.

Phytochemicals in 5 HongJam-powders

Silkworms consume mulberry leaves which contain large amounts of various phytochemicals, absorb phytochemicals from intestines, and store them into tissues such as silk glands and hemolymphs ({Choi *et al*., 2017; Ji *et al*., 2019). Comparing the amounts of phytochemicals present in 5 types of HongJam, while DDS had significantly less flavonoids or KOJ had significantly more, but DBJ and KGJ had no difference compared with WJ (Table 5, $F_{(4, 14)} = 155.557$, $P < 0.001$). The amounts of polyphenol in four types of HongJams were significantly less than that of WJ (Table 5, $F(4, 14) = 21.939$, $P < 0.001$). The amounts of total phytochemicals in types of HongJams were significantly less than that of WJ (Table 5, $F_{(4, 14)} = 95.097$, $P < 0.001$,). Our results suggested that quite amounts of phytochemicals present in 5 types of HongJams might be major factors for acting as antioxidants, increasing neuronal cell survival, and enhancing mitochondria functions. Another reported health benefits of HongJam was inhibiting the onset of Rotenone-induced Parkinson's disease (PD) in animal models (Mai *et al*., 2022; Nguyen *et al*., 2016; Park *et al*., 2022). A recent study reported that higher consumption of flavonoids by PD patients was likely to reduce the risk of mortality (Zhang *et al*., 2022). In addition, one of biochemical basis of enhancement of neuronal cell survivals and memory enhancement effects by HongJam was increased mitochondria complex activities (Kim *et al*., 2019; Mai *et al*., 2022; Nguyen *et al*., 2020b; Nguyen *et al*., 2021). Taken together, phytochemicals in HognJams are important health-enhancing contributors.

Higher pepsin digestibility of HongJams

Since HongJams compose ~70% protein, pepsin digestibility was measured to determine the digestibility and absorption rate of 5 types of HongJams (Table 5). Compared to pepsin

Table 5. Phytochemicals in five HongJams and pepsin digestion rates of them

Phytochemicals (mg/100g)	Silkworm varieties				
	WJ (BOJ)	DBJ	DDS	KOJ	KGJ
Flavonoids	117.3 ± 0.84[a]	129.3 ± 5.22[a]	97.5 ± 0.88[b]	130.1 ± 2.41[c]	122.4 ± 2.40[ac]
Polyphenols	296.8 ± 1.71[a]	266.6 ±3.60[b]	221.7 ± 0.98[c]	258.6 ± 2.98[d]	235.4 ± 1.11[e]
Total	414.1 ± 2.25[a]	395.9 ± 6.96[b]	319.2 ± 1.83[c]	388.7±2.43[b]	357.8± 3.26[d]
Pepsin digestion rate (%)	52.70 ± 0.269[a]	54.66 ± 1.111[a]	49.97 ± 0.092[b]	54.17 ± 0.298[a]	52.95 ± 0.229[a]

Statistical differences were determined by one-way ANOVA followed by HSD post hoc analysis.
[a, b, c, d, e] Different letters indicate significant differences at $P < 0.05$.
The cells with significant differences compared to WJ were shaded.

digestibility of WJ, only DDS had lower pepsin digestibility (Table 5, $F_{(4, 14)} = 95.097$, $P < 0.001$). However, the pepsin digestibility difference between WJ and other HongJams were only 0.25 ~ 2.73%, suggesting that 5 types of HongJams will be digested and absorbed well.

Conclusion

In order to efficiently make HongJam with various functionalities, it is better to use silkworm varieties that weigh higher mature silkworms. Comparing the weight of mature silkworms of silkmoth varieties making white cocoons, recently established Dodam-silkworm, Geumokjam, and Daebaekjam varieties were heavier than that of White-Jade. The results of the nutritional analysis of 5 types of HongJam revealed that the protein and amino acid components of DBJ were most similar to WJ. There were no severe nutrient differences enough to cause a nutritional imbalance between the 5 types of HongJams. These results confirmed that Kumkangjam, which has the heaviest larval weight, is the most optimized variety for efficient production of HongJam, and Daebaekjam is the optimal variety for protein supply.

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