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Effect of cold plasma treatment on the quantitative compositions of silkworm powder

You-Young Jo[1], YoungWook Seo[2], Young Bo Lee[3], Seong-Ryul Kim[4], and HaeYong Kweon[1*]

[1]*Sericultural and Apicultural Materials Division, National Institute of Agricultural Science, RDA, Wanju-gun, 55365, Republic of Korea*
[2]*Postharvest Engineering Division, National Institute of Agricultural Science, RDA, Jeonju-si, 54875, Republic of Korea*
[3]*Technology Services Team, National Institute of Agricultural Science, RDA, Wanju-gun, 55365, Republic of Korea*
[4]*Agricultural Exports Division, Rural Development Administration, Jeonju-si, 54875, Republic of Korea*

Abstract

Atmospheric-pressure plasma technique is a technology for sterilizing agricultural product. In this study, dielectric barrier discharge plasma was applied to silkworm powder for 1 to 5 h with less than 2 ppm of O_3 and NO_2. Quantitative compositions including proximate contents, mineral and heavy metal contents, fatty acids, vitamins, and DNJ contents were measured. Proximate contents of silkworm powder were protein (57.2%), fat (9.9%), fiber (4.6%), ash (10.1%), and moisture (5.7%). These compositions were not affected by the treatment of plasma. Silkworm powder has 5 abundant minerals potassium (K), phosphorus (P), sulfur (S), calcium (Ca), and magnesium (Mg). Among these minerals, plasma treatment decreased the contents of P and S sharply from 732.3 to 176.8, and 492.7 to 185.2 mg/100g, respectively. Heavy metal contents including lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg) were not detected in the silkworm powder. Five vitamins such as ascorbic acid (13.6 mg/100g), riboflavin (5.4 mg/100g), β-carotene (1.8 mg/100g), niacin (0.6 mg/100g), and thiamine (0.4 mg/100g) were not significantly changed by plasma treatment. Silkworm powder is composed of 30 parts saturated fatty acids and 70 parts unsaturated ones. The fatty acid composition was not significantly changed by plasma treatment. The DNJ content of silkworm powder (3.72 mg/g) was also nearly constant within the experimental condition of plasma treatment.

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Introduction

Silkworm (*Bombyx mori* L.) is an industrial insect known to be mass-reared on a greater scale because of its cocoon being utilized for the textile industry. Also, several studies have shown that silkworm powder has been developed to be used as a functional food resource due to its anti-diabetic effects (Chung *et al.,* 1997; Ryu *et al.,* 1997; Ryu *et al.*, 2002).

Moreover, silkworm is an export item among the Korean sericulture products which requires sterilization for the purpose of stability during distribution. However, there are no reports about the qualitative properties of silkworm powder during sterilization and distribution.

Sterilization uses ethylene oxide fuming, irradiation, steam heat sterilization, and ultraviolet (UV) treatments to decontaminate undesirable microorganisms in agricultural

***Corresponding author.**
HaeYong Kweon (Ph. D)
Sericultural and Apicultural Materials Division, National Institute of Agricultural Science, RDA, Wanju-Gun, Republic of Korea
Tel: +82-63-238-2872 / FAX: +82-63-238-3832
E-mail: hykweon@korea.kr

products (Schweiggert *et al.,* 2007). Ethylene oxide has been used for the longest period to inhibit microbes effectively but prohibited in many countries due to carcinogenicity (Fowles *et al.,* 2001; Schweiggert *et al.,* 2007). Farkas (1998) reported that gamma irradiation is also effective to decontaminate various species. Hot steam treatment is useful, but it undergoes undesirable sensory and nutritional changes (Moisan *et al.,* 2001). Although steaming is expensive, the treatment is usually applied before packing the dried silkworm powder.

Plasma is a technique for sterilizing agricultural and food products including rice (Kim *et al.,* 2018b). Cold plasma causes sterilization through damaging the microbial membrane and cell component (Kim *et al.,* 2014). In this regard, plasma is considered as an effective, economical, and environmentally friendly method for critical cleaning. Oxygen species including ozone, and ionized ozone created in the plasma carried out cleaning action (Panjak *et al.,* 2014).

The basic information about the plasma treatment of the silkworm powder was determined from the study. The general composition, notably the mineral contents, heavy metal contents, vitamins, and fatty acid composition of silkworm powder was examined after the treatment with cold plasma.

Materials and methods

Silkworm powder

The silkworm powder (*B. mori* L.) was purchased from MokGolNongJang, a sericulture farm (YeongDeock, Korea). The 3-day-old 5th instar larva were quickly frozen within the liquid nitrogen and lyophilized. Silkworm powder was obtained using roller mill (Kim *et al.,* 2018a).

Dielectric barrier discharge plasma system and treatment

The dielectric barrier discharge (DBD) plasma system (Fig. 1) consists of a plasma actuator (in-house system by the Plasmapp Co. Ltd., Daejeon, Korea). Further, it has a 3 discharge cylinder which has coils inside and outside between 2 mm thick ceramic cylinder, diaphragm pump (20RNS, G&M Tech Inc., Gunpo, Korea) for gas circulation through a Teflon pipe, and silkworm powder container within a 90 L test chamber. Gas concentration

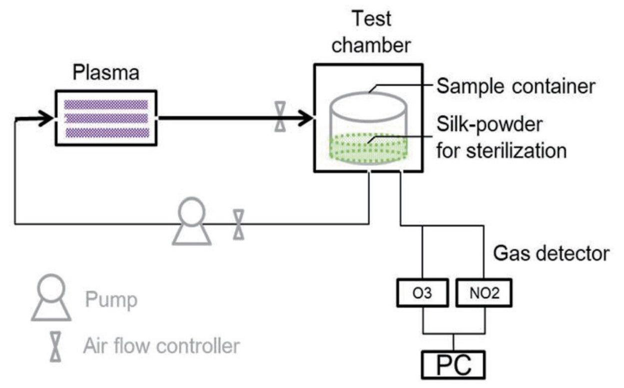

Fig. 1. Schematic diagram of silkworm powder sterilization system based on atmospheric pressure dielectric barrier discharge (DBD) cold plasma technique

was measured using O_3 detector (106-L, 2B Technologies Inc., Colorado, USA) based on UV absorption at 254 nm in the rage of 0 – 100 ppm resolution and NO_2 detector (ENW, Aeroqual Ltd., Auckland, NZ) based on gas sensitive electrochemical (GSE) technology in the range of 0 – 1 ppm with 0.005 ppm resolution. Measured concentration of O_3 and NO_2 was both < 2 ppms. The system was considered as atmospheric pressure plasma system, which generated plasma between two electrodes that were covered with dielectric layers (Moreau *et al.,* 2008). The plasma forming gas flows 15 L/min, which was controlled by a gas mass flow rate controller (DFG-6T, 3-20LPM, Darhor Techonology Co., Ltd., China).

The sample was treated with DBD plasma for 0, 1, 3, and 5 h. Each sample was placed inside the DBD plasma system. The treated sample was used for composition analysis.

Measurement of protein concentration

Proximate analysis of the silkworm powder was determined as follows: The water content was obtained by drying the powder at 105 °C under atmospheric pressure. The amount of crude protein was determined by semi-micro-Kjeldahl method using an automatic protein analyzer (Kjeltec 2400 AUT, Poss Tecator, Mulgrave, Australia). The crude lipids in the dried samples were extracted by diethyl ether and then quantified using a Soxhlet extraction system (Soxtec System HT1043 extraction unit, Foss Tectator, Hoganas, Sweden). The amount of crude fibers was analyzed by 1.25% H_2SO_4 and 1.25% NaOH digestion methods. The amount of crude ash was determined by a dry ashing method at 600 °C.

Mineral contents in silkworm powder were determined using the protocol from the Association of Official Analytical Chemist (AOAC, 1990). Pre-incinerated samples in crucibles were incinerated at 600 °C for 2 h. After cooling, 0.5 g of sample was mixed with 10 mL of 50% HCl and incubated overnight before being filtered through No. 6 filter paper (GE Healthcare Life Sciences, Chicago, IL, USA) with hot water. The mineral contents in the prepared samples were analyzed using an inductively coupled plasma optical emission spectrometer PerkinElmer Optima 8300 (Perkin-Elmer Corporation, Norwalk, CT, USA) by measuring the wavelength and intensities of specific emitted radiant rays for each mineral.

Heavy metal contents in silkworm powder were analyzed following the guidelines from the Ministry of Food and Drug Safety (KFDA, 2001; Kweon *et al.,* 2012). Briefly, sample 2 ~ 3 g was pretreated with 62% nitric acid and hydrogen peroxide and then hydrolyzed through Microwave digestion system (ETHOS, Milestone, Italy). The contents of heavy metal were analyzed with ICP/MS (Agilent Technologies 7500A). Mercury was analyzed in a Mercury analyzer (DMA 80, Milestone, Italy) with same condition with that of Kweon *et al.* (2012).

The 1-Deoxynojirimycin (DNJ) was measured according to the method reported by Kim *et al.* (2003). It was extracted from 0.1 g of dried sample in which it was intensely stirred 2 times for 15 s in the 10 mL 0.05 M HCL solution and diluted with 100 mL water. After adding FMOC(9-fluorenylmethyl chloroformate), quantification was conducted by a high performance chromatography.

Vitamins were analyzed according to the method of Kim *et al.* (2017). Analytical standards of thiamine, riboflavin, nicotinic acid, and nicotinamide, were purchased from Sigma-Aldrich (St. Louis, USA). Sample for thiamine analysis was prepared after extraction with trichloroacetic acid, hydrolyzation, and finally incubation with 300 mg Takadiastase for 18 h. Sample for riboflavin analysis was prepared after treatment with 0.1 N HCl for 60 min, incubation with 300 mg Takadiastase for 18 h, and then purification with 0.2 um syringe filter. Sample for niacin analysis was prepared after treatment with 0.1 N HCl for 60 min, incubation with 500 mg bromelain and 500 mg α-amylase for 3 h at 40°C, and then purification with 0.2 um syringe filter. The prepared sample was injected into LC-MS/MS (Shiseido Nanospace SI2 and API 3200, ABSciex, California, USA) and then calculated. The operation condition for vitamin analysis was same with Kim *et al*. (2017).

Results and Discussion

General composition

General composition of silkworm powder is shown in Table 1. The average crude protein, fat, fiber, ash, and moisture content of silkworm powder is 57.22%, 9.91%, 4.61%, 10.10%, and 5.72%, respectively. The proximate composition of silkworm powder was not significantly affected by plasma treatment except moisture content.

Ryu and Chung (1998) reported the general compositions of silkworm powder itself and mulberry tree. The general composition of silkworm powder is protein (56.76%), fat (9.27%), fiber (6.62%), ash (9.14%), and moisture (4.77%). The common edible plant, *Morus alba*, Chungilppong is composed of protein (24.23%), fat (2.65%), fiber (9.56%) ash (9.79%), and moisture (9.13%). By comparison of the general composition between silkworms and mulberry leaves, protein and fat in silkworm powder is higher than those in mulberry tree. Protein

Table 1. Proximate composition of silkworm powder treated with plasma

Treatment condition	0 h	1 h	3 h	5 h	average	Silkworm powder*	Mulberry Leaves*
Moisture (%)	4.52	5.05	6.18	7.12	5.72±1.16	4.77	9.13
Ash (%)	10.22	10.2	10.01	9.96	10.10±0.13	9.14	9.79
Protein (%)	57.79	57.59	56.74	56.76	57.22±0.55	56.76	24.23
Fat (%)	9.7	10.29	10.02	9.62	9.91±0.31	9.27	2.65
Crude fiber (%)	4.77	4.64	4.49	4.53	4.61±0.13	6.621	9.56

*Ryu and Chung (1998)

concentration of livestock including beef, pork, and chicken is 20-30% (Rural Development Administration, 2011; United States Department of Agriculture, 2015). By comparison, silkworm powder is 57.22%, and had a much higher protein concentration therefore, a valuable and potential source of dietary protein and can be used as a protein supplement.

Mineral content

Table 2 shows the mineral content of the silkworm powder. Among the 10 minerals examined, five minerals specifically potassium (K), phosphorus (P), sulfur (S), calcium (Ca) and magnesium (Mg) had high concentration of over 100 mg/100g as follows: K 2769.57, P 732.31, S 492.7, Ca 449.89, and Mg 345.34 mg/100g, respectively. On the other hand, other minerals such as sodium (Na), iron (Fe), zinc (Zn), manganese (Mn), and copper (Cu) were found less than 100 mg/100g as follows; Na 57.88, Fe 6.05, Zn 4.66, Mn 4.42, and Cu 1.48 mg/100g, respectively. According to the plasma treatment, the mineral concentration of silkworm powder did not significantly changed except at 5 h treatment. Treatment with plasma for 5 h decreased Phosphorus and Sulfur sharply from 732.31 to 176.83, and 492.7 to 185.2 mg/100g.

Ji's group reported that matured silkworm has 5 abundant minerals such as K, P, Ca, S, and Mg (Ji *et al.,* 2016a; Ji *et al.,* 2016b). Cha *et al.*(2010) also published silkworm has some abundant minerals like K, Ca, and Mg. The results corroborated the findings of other researchers.

Table 2. Mineral concentration (mg/100g) of silkworm powder treated with plasma

Treatment condition	0 h	1 h	3 h	5 h
Ca	449.89	417.92	409.68	446.29
Cu	1.48	1.04	1.00	0.92
Fe	6.05	6.14	5.94	7.09
K	2769.57	2463.25	2569.34	2718.33
Mg	345.34	320.34	313.85	341.55
Mn	4.42	3.99	4.06	4.31
Na	57.88	143.04	47.41	50.84
P	732.31	678.68	666.66	176.83
Zn	4.66	5.51	3.77	4.23
S	492.7	501.7	451.5	185.2

Heavy metal content

Table 3 shows the heavy metal contents of silkworm powder indicating the absence of lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg). As expected, plasma treatment did not induce any changes of heavy metal contents. Matured silkworm powder also reported no detection of heavy metal (Ji *et al.,* 2016a; Ji *et al.,* 2016b). The results therefore showed that the silkworm was not contaminated with heavy metals.

Vitamin content

The vitamin contents of silkworm powder yielded ascorbic acid (13.56 mg/100g), riboflavin (5.37), β-carotene (1.76), Niacin (0.61), and thiamine (0.4) (Table 4). Although, silkworm powder was treated with plasma, the vitamin contents were not significantly changed.

Fatty acid content

The silkworm powder had high amount of lipid as shown in Table 5 with14 fatty examined. The ratio of saturated and unsaturated fatty acids was about 30:70. The detected saturated fatty acids were palmitic acid (C16:0, 20.42%), stearic acid

Table 3. Heavy metal concentration (ppm) of silkworm powder treated with plasma

Treatment condition	0 h	1 h	3 h	5 h
Pb	ND	ND	ND	ND
Cd	ND	ND	ND	ND
As	ND	ND	ND	ND
Hg	ND	ND	ND	ND

ND: not detected

Table 4. Vitamins contents (mg/100g) in silkworm powder treated with plasma

Treatment condition	0 h	1 h	3 h	5 h
β-carotene (A)	1.76	1.77	1.66	1.82
Thiamine (B_1)	0.4	0.36	0.34	0.37
Riboflavin (B_2)	5.37	5.1	4.04	4.52
Niacin (B_3)	0.61	0.56	0.54	0.5
Ascorbic acid (C)	13.56	15.23	13.65	17.87

Table 5. Fatty acid compositions of silkworm powder (% of total fatty acids)

Treatment condition	0 h	1 h	3 h	5 h
Palmitic acid (C16:0)	20.42	20.39	20.49	20.41
Palmitoleic acid (C16?:1n7)	0.46	0.5	0.52	0.54
Stearic acid (C18:0)	9.54	9.68	9.58	9.52
Oleic acd (C18:1n9)	21.48	21.39	21.52	21.3
Vccenic acid (C18:1n7)	0	0	0	0
Linoleic acid (C18:2n6)	9.53	9.62	9.53	9.56
γ-Linoleic acid (C18:3n6)	0.2	0.07	0.09	0.1
Linolenic acid (C18:3n3)	37.91	37.87	37.75	38.06
Eicosenoic acid (C20:1n9)	0.08	0.14	0.12	0.17
Arachidonic acid (C20:4n6)	0	0	0	0
Eicosapentaenoic acid (C20:5n3)	0	0	0	0
Docosatetraenoic acid (C22:4n6)	0	0	0	0
Decosahexaenoic acid (C22:6n3)	0	0	0	0
Total	100	100	100	100
Saturated fatty acids	30.33	30.42	30.48	30.27
Unsaturated fatty acids	69.67	69.58	69.52	69.73
Mono-unsaturated	22.03	22.03	22.15	22.01
Poly-unsaturated	47.65	47.56	47.37	47.72

(C18:0, 9.54%), and myristic acid (C14:0, 0.37%). Unsaturated fatty acids detected were as follows: linolenic acid (C18:3n3, 37.91%), oleic acid (C18:1n9, 21.48%), linoleic acid (CC18:2n6, 9.53%), palmitoleic acid (C16:1n7, 0.46%), γ-Linoleic acid (C18:3n6), and Eicosanoid acid (C20:1n9). Although silkworm powder was treated with plasma, the total ratio between saturated and unsaturated fatty acids and each fatty acid composition were not significantly changed.

DNJ content

Table 6 shows the DNJ content of silkworm powder. DNJ of silkworm powder was 3.72 mg/g with the content varying from 3.62 to 3.85 mg/g with plasma treatment suggesting the content to be constant with or without plasma treatment.

Table 6. Deoxynojirimycin content in silkworm powder treated with plasma

Treatment condition	0 h	1 h	3 h	5 h
DNJ (mg/g)	3.72	3.85	3.62	3.74

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