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Sterilization effect and fatty acid composition of silkworm powder (*Bombyx mori* L.) by heat treatment

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Abstract

The effect of heat treatment on the sterilization and fatty acid compositions of silkworm powder was carried out under the guidance of Ministry of Food and Drug Safety. Food borne pathogens or microorganisms including *E. coli*, *Salmonella spp.*, *Listeria monocytogenes*, *Staphylococus aureus*, *Clostridium perfringens* and aflatoxin, were not detected. The fatty acid composition was hardly changed after the silkworm powder was treated to a high temperature of 121°C. The low temperature sterilization of silkworm powder at 63°C decreased the concentration of *E. coli* while high temperature sterilization at 121°C has found no traces of microorganism.

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Introduction

Silkworm powder, which is known to help control blood-glucose levels, is lyophilized 3rd day 5th instar of silkworms (*Bombyx mori* L.) and ground into powder. The oral administration of hot-air dried extract of silkworm powder to rabbits resulted to an increased insulin content and the lowering of blood glucose levels were reported (Ryu *et al*., 1997). The mechanism of blood glucose lowering capability of silkworm powder was due to its α-glucosidase inhibitory activity in the small intestine (Ryu *et al*., 2002). The silkworm powder, which is a high-protein food material, containing essential amino acids and unsaturated fatty acids, is not only used for lowering blood glucose levels but also for improvement of liver function and blood circulation (Cho *et al*., 1989; Ikeda *et al*., 1998). It is known that the fatty acids in silkworm powder contains large amount of linolenic acid, oleic acid and palmitic acid. These unsaturated fatty acids are effective for cardiovascular diseases and diabetes and have various physiological activities such as prevention of brain diseases (Siscovick *et al*., 2017; Bazinet and Layé, 2014; Salmerón *et al*., 2001). Linolenic acid, a kind of omega-3 fatty acid, most abundant in silkworm powder, has its known functionalities such as: anti-inflammation (Jung *et al*., 2010; Weldon *et al*., 2007), anti-cancer (Begin and Ellis, 1987; Hori *et al*., 1987), anti-thrombosis (Bang *et al*., 1980; Dyeberg, 1986), prevention of hypertension (Pieters *et al*., 2019), and adaptability to darkness (Lee *et al*., 2014).

Perilla oil is an edible vegetable oil derived from perilla seeds, an omega-3 fatty acid-rich food. However, it is low in oxidative stability and is rancid easily during storage, so unpleasant odors

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are likely to be produced. It is also easily denatured by heat (Kim *et al*., 1994). Silkworm powder containing a large amount of unsaturated fatty acid is mainly distributed or consumed in the form of a powder or a pill and is ready-to-eat food without the need for cooking. The freeze-dried silkworm powder was registered as a health functional food that can help control blood glucose levels (Health functional food functional raw material certificate No. 2009-67). The manufacturing process is prescribed such that 3rd day 5th instar of silkworms (*Bombyx mori* L.) are frozen at -20°C or lower, lyophilized, ground (over 80 mesh) and then sterilized for more than 15 min. The silkworm powder treated with edible enzymes has been reported to have thermal stability as confirmed using TGA and DSC tests (Jo *et al*., 2018). However, the effect of sterilization condition on silkworm powder and its effect on fatty acid composition have not been studied. In this study, sterilization effects and fatty acid composition of silkworm powder by heat treatment were investigated to determine its stability and safety

Materials and Methods

Silkworm Powder

The silkworm powder used in this experiment was made from the lyophilized 3rd day 5th instar of silkworms (*Bombyx mori* L.) which were reared on mulberry leaves (Chungilppong) in sericultural farm (Boeun, Korea) .

Safety verification of silkworm powder

Since the silkworm powder will be considered as ready-to-eat food without cooking, hence its safety was verified through the presence of harmful microorganisms and aflatoxin. The presence or absence of harmful microorganisms such as *E. coli*, *Salmonella spp*., *Listeria monocytogenes*, *Staphylococcus aureus*, and *Closteridium perfringens* were verified according to food standards and specifications. The content of total aflatoxin, a fungal toxin, was also analyzed according to food standards and specifications.

Inoculation of *E. coli*

Escherichia coli were inoculated into silkworm powder assuming coliform contamination might be due to careless handling. *E. coli* (ATCC 1039) was cultured in LB medium (Luria Bertani Broth, Miller, Narae Biotech, Korea) and then CFU (colony forming unit) of the microorganism was measured using a stepwise dilution method. The final *E. coli* suspension was prepared to 10^7 CFU/0.01 mL. The silkworm powder was sterilized by heat treatment at 121°C for 30 min and then inoculated with 10^7 CFU/0.01 mL of *E. coli*.

Verification of sterilization effect

The silkworm powder was divided into the aluminum stand wrapping paper (AL sterilizing stand, Izu-en-Pak, Gwangju, Korea) by separating the *E.coli* treatment and control. Sterilization was carried out according to standards for processed meat products, processing standards of non-heated meat products, and ingredient standards of livestock product proposed by the Food and Drug Administration of Korea (2017). The following sterilization conditions were applied: 63°C for 30 min, 63°C for 60 min, 121°C for 5 min, 121°C for 15 min, and 121°C for 30 min. To verify the sterilization effect, 1 mL of LB medium was added to the 0.1g of sterilized sample, and the mixture was vortexed and centrifuged at 13,000 rpm for 10 s. The resulting supernatant was mixed well with 4 mL of LB medium, and 1 mL of each was added to a new tube, diluted with 4 mL of LB medium, and incubated overnight at 37°C.

Fatty acid composition analysis

Changes in fatty acid composition of silkworm powder by heat treatment under various conditions were analyzed according to AOAC 963.22 (AOAC 2005).

Results and Discussion

Safety verification of silkworm powder

It is known that ingestion of foods that are contaminated by harmful microorganisms cause diseases such as food poisoning. Listeria (*Listeria monocytogenes*), known as food-harmful bacteria, have been reported to cause abortion in pregnant women and cause listeriosis in adults, such as laxity and conjunctivitis (Farber and Peterkin, 1991). *E. coli,*

Table 1. Effect of heat treatment on the food borne pathogens in silkworm powder

Food borne Pathogens	Normal	63°C 30min	63°C 60min	121°C 5min	121°C 30min
E. coli	ND[1)]	ND	ND	ND	ND
Salmonella spp.	ND	ND	ND	ND	ND
Listeria monocytogenes	ND	ND	ND	ND	ND
Staphylococcus aureus	ND	ND	ND	ND	ND
Closteridium perfringens	ND	ND	ND	ND	ND

[1)]Not detected.

Table 2. Effect on the sterilization (heat treatment) of silkworm powder

E.coli inoculation	-	+	+	+	+	+	+
Heat treatment condition Temperature(°C)/Time(min)	-	-	63/30	63/60	121/5	121/15	121/30
The number of detected *E.coli* (CFU/ml)	0	$2.3X10^9$	$1.9X10^9$	$0.8X10^9$	0	0	0

Salmonella, and *Staphylococcus aureus*, which are transmitted by food and water, cause diseases such as food poisoning and salmonellosis by ingestion (Han *et al*., 1969; Ryu *et al*., 2007). Silkworm powder has been used as a functional food material since 1998, and has been consumed directly without being heated. To verify the microbial contamination before the heat treatment (sterilization) of the silkworm powder purchased from the farmhouse, the presence of harmful microorganisms such as *E. coli, Salmonella, Listeria, Staphylococcus aureus* and *Clostridium* was determined. The harmful microorganisms were not detected at all, and thus silkworm powder was found to be safe from harmful microorganisms (Table 1). Mycotoxin, a secondary metabolite of fungi, is also recognized as an important factor in food safety. Among them was a toxin initially named '*Aspergillus flavus* toxin' by the Interdepartment Working Party on Groundnut Toxicity Research in the UK, which was later called aflatoxin (Sargeant *et al*., 1961). Aflatoxin is one of the secondary metabolites of fungi (*Aspergillus flavus, A. parasiticus, A. nominus*, etc.) present in nature and is classified as a controlled substance. It is well formed in tropical and subtropical regions with high temperature (25-30°C) and high humidity (80-85%). It is also well formed during long storage period from harvest to drying and inadequate ventilation. Among the agricultural products, it is known to be formed well in cereals such as rice, barley and wheat, and in carbohydrate-rich substrates such as corn and in nuts such as peanuts, pistachios, walnuts (Gourama and Bullerman, 1995). The Korean herbal medicines, such as *Bombycis corpus*, are tested for aflatoxin B1 and total aflatoxin levels, which are below 10 ug/kg and 15 ug/kg, respectively (Kim *et al*., 2017). Silkworms at the 5th instar stage were infected with *A. flavus* K-199, a silkworm extract was prepared and tested for oral toxicity by feeding which resulted in the death of all silkworms used in the test after 4 d of feeding (Ohtomo *et al*., 1975). An investigation of aflatoxin contamination of agricultural products commonly used in food and medicine found that the main cause of contamination was unsanitary management, more than just the water content (Kim *et al*., 2017). The silkworm powder used in this experiment did not detect aflatoxin, so it was able to verify the safety of the silkworm powder produced in the farmhouse.

Sterilization (heat treatment) effect

The silkworm powder was infected with 1×10^7 CFU of total coliform, assuming contamination due to careless handling during manufacture and distribution of silkworm powder. Each powder was sterilized (heat-treated) under different conditions, and the effect according to sterilization conditions is shown in Table 2. The infected *E. coli* was not completely sterilized and showed 0.8×10^9 CFU after treated for 60 min in the low temperature heat treatment (63°C). On the other hand, at high temperature (121°C), even if treated for only 5 minutes, all the bacteria were killed. The Korea Food and Drug Administration (KFDA) differentiate between heat-processed and sterilized food products. The heat-processed are products that are heat-treated at temperatures of 63°C or higher while sterilized food are products

Table 3. Composition of fatty acid of silkworm powder by heat treatment

Fatty acid \ Heat treatment Condition	Untreated	63°C 30min	63°C 60min	121°C 5min	121°C 30min
Myristic acid (C14:0)	0.1	0.1	0.1	-	-
Palmitic acid (C16:0)	19.4	19.4	19.4	19.7	19.6
Stearic acid (C18:0)	8.6	8.7	8.7	9.5	9.4
Arachidic acid (C20:0)	0.5	0.5	0.4	-	-
Total SFA1)	28.6	28.7	28.6	29.2	29.0
Palmitoleic acid (C16:1)	0.5	0.5	0.4	0.4	0.4
Oleic acid (C18:1)	23.7	23.4	23.4	23.2	23.1
Linoleic acid (C18:2)	7.3	7.2	7.5	7.4	7.6
Linolenic acid (C18:3)	39.8	40.1	39.9	39.5	39.8
Total UFA2)	71.3	71.2	71.2	70.5	70.9
Unknown	0.1	0.1	0.2	0.3	0.1
Sum	100	100	100	100	100

1)SFA: saturated fatty acid. 2)UFA:unsaturated fatty acid.

that are heat-treated at temperatures of 120°C or higher. In case of silkworm powder, high temperature sterilization method is expected to be used to prevent bacteria contamination that might be due to careless handling during the commercialization process.

Fatty acid content of silkworm powder and compositional change by heat treatment

In general, it is known that vegetable oil having a high content of polyunsaturated fatty acid is easily oxidized due to low heat stability (Kim *et al*., 1994). Therefore, to determine heat stability of silkworm powder containing large amount of unsaturated fatty acid, the changes in the fatty acid composition was measured and the results were shown in Table 3. The silkworm powder was composed of less than 30% of saturated fatty acid and more than 70% of unsaturated fatty acid. The main saturated fatty acids were palmitic acid (C16:0) 19.4% and stearic acid (C18:0) 8.6%. The main unsaturated fatty acids were linolenic acid (C18:3) 39.8%, oleic acid (C18:1) 23.7% and linoleic acid (C18:2) 7.3%. The content of saturated fatty acid was not changed by the low temperature treatment (63°C). The content of oleic acid, unsaturated fatty acid, was slightly decreased from 23.7% to 23.4%, but no significant change was observed. On the other hand, in the case of high temperature (121° C) treatment, the proportion of total saturated fatty acid slightly increased from 28.6% to 29.1%, and the total unsaturated fatty acid decreased from 71.3% to 70.7%. Saturated fatty acid increased stearic acid from 8.6% to 9.5%, palmitic acid slightly increased from 19.4% to 19.7%, but myristic acid and arachidic acid were not detected. In unsaturated fatty acids, there was a slight decrease in palmitoleic acid, oleic acid and linolenic acid, and a slight increase in linoleic acid. Silkworm powder is rich in linolenic acid, an omega-3 fatty acid that modern people may lack of. The main functions of omega-3 fatty acids are anti-inflammatory (Jung *et al*., 2010; Weldon *et al*., 2007), anti-cancer (Begin and Ellis, 1987; Hori *et al*., 1987), anti-thrombotic (Bang *et al*., 1980; Dyeberg, 1986), prevention of hypertensive (Pieters *et al*., 2019) and adaptability to darkness (Lee *et al*., 2014). In general, it is known that perilla oil, which contains a lot of vegetable omega-3 fatty acid (60%), is low in oxidative stability and rancid easily during storage, so unpleasant odors are likely to be produced (Kim *et al*., 1994). In the case of hempseed (*Cannabis sativa* L.), the unsaturated fatty acid content were increased by roasting but there has no significant change in the proportion of fatty acids. The increased unsaturated fatty acids were 3% for oleic acid, 3% for linoleic acid, and 4% for linolenic acid (Jang *et al*., 2018). Oh *et al*. (1991) reported that the retort sterilization effect on the components of red and white fish showed little change in fatty acid composition at 98°C heat treatment, but the unsaturated fatty acid gradually decreased and saturated fatty acid increased at 121°C treatment. In general, fatty acids are known to decrease the proportion of unsaturated fatty acids and increase the proportion of saturated fatty acids by thermal oxidation (Lim *et al*., 2017). As for the fatty acid of silkworm powder, there was a difference in the increase and decrease according to the type of fatty acid by heat treatment, but no significant difference was found. This result is consistent with the general fatty acid tendency. Taken together, the treatment of silkworm powder at 121°C for 30 min does not significantly affect the proportions and stabilities of various fatty acids in silkworm powder, but completely eradicated harmful microorganisms.

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