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Multi-functions of *Protaetia brevitarsis surensis* (PBS, Larvae for Food) *Reported* in South Korea

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Nowadays most people in advanced countries endangered under adult diseases such as hypertension, diabetes, hyperlipidemia, inflammatory disease, human oxidation, and liver-related diseases. In South Korea also very serious situation for the adults and children by the changing from vegetable food to animal food and changed labor pattern from physical labor to mental labor.

We aim to review *Protaetia brevitarsis* surensis (PBS)'s beneficial multi-functions on human with research reports and verification of well-known common sense in South Korea, which was commonly known as medicinally very effective in liver related diseases. PBS larvae known as cold body characteristics, so, it more effective to Yang-in(Oriental physical constitution classification method: Warm body which Jinseng, *Panax ginseng*, not available people) than Um-in(Cool body-Jinseng available people). The functional effects mostly known effective on liver related diseases mainly by oriental clinic and as folk remedies. However, nowadays PBS larvae's folk remedies and other multi-functions scientifically researched and that results verified by many papers such as whitening of skin therapeutic effects, prevention of breast cancer, inflammatory disease, human oxidation, and liver-related diseases. For the increasing of medicinal effects, advanced

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fermentation and ethanol extraction methods will be very useful compared to non-fermentation of PBS larvae and water extraction. So, we concluded that PBS could diminish or decreasing the adult diseases and it will be very useful food and medicinal materials simultaneously for the human food and medicine by the advanced extraction methods.

Keywords: Protaetia brevitarsis surensis (PBS); multi-function; medicinal materials; insect food.

1. GENERAL INTRODUCTION AND OBJECTIVES

1.1 General Introduction

In previous paper, we introduced that the historical characteristics of PBS [1] and oviporation characteristic [2] and other PBS larvae's feed related materials and general fermentation methods. However, for the PBS farmers sell the PBS larvae very low price, such as $30-40 per raw larvae 1 kg (year '20~'21), which price is very low for getting profit for the small scale farmers and if continuing this price level, PBS farmers will disapper near soon except big smart factory established company. So, small scale most farmers want to sell enhanced price not supporting raw PBS's larva only but selling they own after processing, which basically improve price highly but selling scale is very limited which is the limiting factor to selling broadly. Additionally, getting processing permission is very difficult in South Korea. So, we want to support the economical viable processing methods for the farmers and give many information of good functionality for consumers which might be broadening the basic knowledge and attractive to eating. In South Korea, Government trying to 6th Industry in agricultural part, which means 1st Industry for Growing or Producing of agricultural materials, 2nd Industry for processing of agricultural products, which mostly getting HACCP (Hazard Analysis Critical Control Point, which means a preventive food safety management system to prevent hazard, Welcome to the Korea Agency of HACCP Accreditation and Services(KAHAS).) certification, and 3rd Industry for Services and marketting, which mostly focused on selling by Internet, person to person, TV home shopping and etc. That 1st + 2nd + 3rd = 6th which is new agricultural paradigm in South Korea started from 2010s.

1.2 Objectives

Nowadays most of advanced people endangered under adult diseases such as hypertension, diabetes, hyperlipidemia, inflammatory disease, human oxidation, and liver-related diseases.

Additionally, the global warming is very serious problem for maintain or living humans in this earth. In point of human food, most livestock such as cattle, chicken, pig and et cetra support animal nutrients greatly and we are living with health, however, the animal needed 5-12 times more plant feed compared to insect larvae. The more needed plant feed bring about the global warming by using of machinery, fertilizers, and agricultural chemicals.

However, the nutrients and functional effects of food insect not well published worldwide but just published simple part and just known within some people of South Korean. So, in this paper, we want to inform to the worldwide people by supporting the nutritional efficiency of insect larvare, especially *Protaetia brevitarsis* surensis because, which is most popular human's food insect. So, this information could support variable effective information to agro-food researchers and consumers together.

2. INTRODUCTION AND OBSERVATIONS

2.1 General Introduction

Cho and Cho [1] introduced that the history and general effects of PBS in broadly. Additionally, Suh and Kang (2007) also introduced that in view of the insect family which adult named as the white spotted flower chafer, *Protaetia brevitarsis* surensis, part of the family of Scarabaeidae belonging to the order Coleoptera PBS primarily found in Eastern Asia.

There are many papers published and introduced the PBS larvae's nutrient components and many of them, Noh etal, [3] introduced that the PBS larvae's nutrition and change of medicinal effects by the feed sources during starvation of larvae. The starvation is very important for removing the compost smell by feeding of fermented saw-dust of oak tree or others feed sources. Additionally if no starvation the smell is very serious bad, so most farmers basically doing the starvation for

four to 5 days washing 2-3 times per day with tap water, however, it reducing the larvae's body weight about 25-30%, which is very disliking point and economically unviable for the raw body selling farmers. So, Noh et al researched that how can diminish the weight loss and improve the functionality. That results are summarized that 1) the chemical composition, fat, and amino acids of the *Protaetia brevitarsis* seulensis (Kolbe; PBS) larvae according by the type of food sources, and It was not feed for four days. 2) The weight loss increased in the order of food pumpkin, aloe, rice bran and fasting with 0, 2.8, 15 and 27%, respectively. In all feeding larvae, known as 56% of crude protein it was ranged of 50.7 ~ 55.4% more or less broadly and fat content was 16.1 ~ 18.5%. The rice bran fed larvae showed highest content of both crude protein and fat. Unsaturated fatty acid content of the total fatty acid was in the range of 76.4 to 75.5%. The highest unsaturated fatty acid content found in the aloe fed larvae. Among the structural amino acids, essential amino acid was 36% and non-essential amino acids was 34%. The amount of total polyphenol were 23.57, 23.38, 23.05 and 22.7 mg g-1 in the larvae feeding bran, aloe, pumpkin, and fasting, respectively. ABTS, DPPH radical scavenging activity was the highest in the rice bran fed larvae with 17.94 mg TEg-1 and 2.00 mg TEg-1. The L value indicating the lightness of larvae was 72.3, 64.7, 58.3 and 44.3 in the larvae feeding pumpkin, aloe, rice bran and nothing, respectively; the pumpkin fed one was the most bright but fasted larvae was the most dark. Finally, they concluded that feeding fruit or vegetables more valuable and economically viable with multifunctions than just fasting the PBS larvae before processing in hot water and drying for washing and disinfection.

At present, the functional effects of PBS researched and well known as anti-bacterial protein, care of liver damage or disease, anti-cancle effects [4,2,5-9].

2.2 General Components and Harmful Materials of PBS larvae

For the general nutrient compositions were some different by the researchers, that reasons were mostly different PBS larvae's sample which means starvation degree and sampling stage were more or less variable. However, we introducing the general research results in this paper :

Chung et al. [10] published that the general nutrients of PBS larvae's powder such as moisture, crude protein, crude fat, crude ash, crude fiber, and carbohydrates showed that the content of crude protein, fat, fiber, and ash were 57.9±0.01%, 16.6±1.81, 5.31±0.10 and 8.36±0.10%, respectively. They also analyzed the components of amino acids, which were composed of 17.7% essential and 34.0% non-essential. The unsaturated fatty acid content was 61.1%, which was mostly oleic acids. Additionally, the minerals contained highly in body organization, such as K, P, and Mg with 1,597, 724, and 366 mg/100 g, respectively. They analyzed and evaluated PBS larvae's body by the lysophilized powder, which also confirmed that all bacteria and all heavy metals analyzed. The very small amount of Hg (0.1±0.042 mg/kg) were detected below the permission level and additionally it also not exceeded in the harmful heavy metals of Ministry of Food and Drug Safety (the limitations = Pb, 0.3; Cd 0.1; As, 0.1 mg/kg). In our non-published research, the toxicity not found for food within 5g per day for more than one year, which was tested for more than 100 people in South Korea. However, following case could making toxicity and diarrhea if breeded in bad environmental condition (temperature, oxyzen content, humidity) and contaminated or larva's feed contained above amounts of heavy metals.

2.3 Major and Minor Components

The component of fatty acids is very important in animal because most animal contain high amount of saturated lipid, which positively related with blood related disease, especially meat eating country people. So, in this paper I introducing fat related research results in PBS larvae. Some of fat related paper, Yeo et al [11] published that the 48 volatile oils (fatty acid composition) of PBS's larvae and the analyzing methods. Which are summarized that volatile oils were identified form PBS's larvae by gas chromatography/mass spectrometry (GC/MS). They detected that 48.7% acids as the major group in PBS larvae which was the largest proportion of the volatile compounds and other components esters, hydrocarbons, alcohols, miscellaneous, aldehydes and terpenes were 19.8, 18.9, 8.37, 1.71, 1.35, 1.16%, respectively. The major volatile constituents were 9-hexadecenoic acid (16.75%), 6-octadecenoic acid (14.88%) and n-hexadecanoic acid (11.06%). They analyzed the composition of fatty acid with GC analysis and 16 fatty acids identified. The characteristic of the fatty acid profile of PBS larvae was the greatest amounts of unsaturated fatty acid (good for human health) proportion was

80.5% which was more or less different to the previous research results, however, saturated fatty acids was 19.5% of total fatty acids. The classification by the number of carbon and double bonding number' oleic acid(C18:1) was the highest with 64.24% and then followed by palmitic acid(C16:0), palmitolic acid(C16:1), and moleic acid, which were palmitic acid(C16:0,), palmitoleic acid(C16:1), and linoleic acid(C18:2) with 15.9, 10.4, and 4.69%, respectively.

2.4 Extraction Methods

Extraction method of PBS's larvae: nowadays, analyzing machine is very developed such as LC, GC, ICP and others, however, there was no pretreatment and analyzing methods was established in the PBS larvae. So, I would like to we introduce that with research reports: The 1st report by Sung et al. [12] published that anti-inflammatory and whitening effects of PBS larvae extracts by oriental conversion methods. The experimental methods were followed by PBS larvae's extracts and the methodologies were 1) fresh, roasting time 1, 2, 3 times and 2) steamed and after steaming, 3) extracting method which was the most valuable in 80% ethanol and the result the 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities was 85.5.

However, another methodology for the PBS larvae analysis was introduced by Kim and Cho [13]. The production of protein hydrolysate (HD) from PBS larvae and the results summarized that the additional treatment was high pressure (100 MPa, 50° C, 24 hr, complex enzymes) in HD. The enzymatic treatment and ultrasonic treatment (30 min.) produced more high soluble solid content, extraction yield, total protein content, protein yield, protein yield with low molecular weight than in the hydrolysates produced by elimination of high pressure hydrolysate single treatment.

2.5 Major or Minor Feeds for Larvae Change the Larvae's Multi-functions

Another effective PBS larvae nutrients was published by Noh et al [3]. The nutrients and multifunctional components varied during fasting and that results were introduced before, however, Kim et al. [14] researched the major components between fermented oak sawdust (FOS) or spent mushroom substrates (SMS, *Pleurotus eryngii*) for making PBS larvae's feed and published that results and summerized as following: 1) the feeding major source effects on the antioxidation activity. 2) Total polyphenol content was 32% higher in extracts of larvae fed on SMS (P. eryngii) (75.33±0.43 mg GAE/g) than in extracts of larvae fed on FOS (57.02±1.73 mg GAE/g). 3) The flavonoid content of extracts of larvae grown on FOS and SMS (P. eryngii) was 24.6±0.28 mg/g and 25.4±0.75 mg/g, respectively. 4) DPPH radical scavenging activity increased in an extract concentration-dependent stream, and the DPPH radical scavenging capacity of the extract of larvae produced on SMS (P. eryngii) was higher than that of the larvae produced on FOS. 5) The reducing power of the larval extracts produced on FOS and SMS (P. eryngii) increased in an extract concentration-dependent manner, but there was no significant difference between them. 6) The extract of larvae fed on SMS (P. eryngii) (66.55±0.99 uM TE/ g) had a higher oxygen radical absorbance capacity (ORAC) than extracts of larvae grown on FOS (76.32±0.48 uM TE/g).

3. MULTI-FUNCTIONS OF PBS LARVAE AND EXPERIMENTAL METHODS

3.1 Antithrombic Efficiency

Choi et al. [15] published that the effect of antithrombotic efficacy of PBS larvae additionally which can reducing high blood pressure which research results were summarized the following contents : 1) The antithrombotic efficacy of PBS larvae extract during 21 days. 2) Rats (SPF rat, weight 240~260 g) were divided into 16 groups (5 rats per group), they were: control group and PBS larvae's extract groups with dose of 0.1, 0.5, 1, 2.5, 5, 10, 25, 50, 75, 100, 200, 250, 500, 750, 1,000 mg/kg kg of body weight. 3) Thromboplastin time (PT) and activated partial thromboplastin time (aPPT) as antithrombotic efficacy tested in this animal experiment at 7, 14, 21 days. 4) Overall, the administration dose of PBS larvae extract over 50 mg/kg at 7, 14 and 21 days for PT and over 25 mg/kg at 7, 14 and 21 days for aPPT tented to be longer than that of other groups. 5) In addition, the optimal administration doses of PBS larvae extract to improves antithrombotic efficacy were 75, 100, 200 and 250 mg/kg at 7, 14 and 21 days for PT ($p<0.05$) and 50 and 100 mg/kg at 7 days, 75 mg/kg at 14 days, or 50, 100, 200 and 250 mg/kg at 21 days for aPPT ($p<0.05$). It can be concluded that PBS larvae extract at optimal levels have antithrombotic efficacy.

3.2 Therapeutic Effects

They also introduced that the PBS larvae's therapeutic effects in the treatment and prevention of breast cancer, inflammatory

disease and liver-related diseases such as hepatic cancer, liver cirrhosis, and hepatitis [16,17]. High antioxidant effect of PBS larvae at different growth stages were reported [14,18] and the antimicrobial peptides protaetins have been purified from PBS larvae [19].

3.3 Therapeutic Effects' Limitation Case

However, the effects were not available on the therapeutic effect on carbon tetrachloride (CTT) or ethanol induced hepatotoxicity (EHT; [6]), which paper results summarized following : 1) PBS larvae's extract could protects or modulates the liver injuries induced by carbon tetrachloride or ethanol in Sprageue-Dawley rate. 2) The results concluded that PBE has not appreciable therapeutic effect on CTT or ETH. They experimental methods and materials were abbreviated as following: 1) Rats was used instead of human body as induced by the treatment of CTT or ETH which were manifested by the observation of the significant changes in liver weight, serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities, serum thiobarbituric acid reactive substances (TBARS), and microsomal detocification enzymes(cytochrome P_450), cytochrome b55, and cytochrome b55 reductase). 2) The effect of PBF on the liver damage induced by the chemicals evaluated with the extent modulated in change of biochemical parameters above. 3) Exposure to ethanol alone resulted a significant change in the ration of liver per body weight, ALT activity, and microsomal detoxification enzymes (cytochrome P_450, cytochrome b55, and cytochrome b55 reductase), but did not significantly changes in the levels of serum AST activity and TBARS. 4) Pretreatment coith PBE did not modulate the alteration of the ratio of liver to body weight, and the activities of serum aminotransferases (AST. ALT), TBARS, and microsomal detoxification enzyme (cytochrome p_450, cytochrome b55, and cytochrome b55 reductase.

3.4 Antioxidation and Anti-mutationtion Effects

The third part papers summarized as : Han et al., [20] introduced that *Cordyceps militaris* which is known as cordycepin and its effects were antioxidation [21] and anti-mutationtion [22,23]. Park et al [24] investigated antioxidative and nitrite scavenging activities of *Cordyceps militaris* and published that effects. For the methodology of extraction, they compared water and ethanol for the extraction in 4 levels of pH (1.2, 3.0, 4.2, 6.0). The cultured that the fruiting body and mycelia of artificially cultivated it, which extracted with water and 70% ethanol. In the results, ethanol extracted in low pH (1.2) were more higher nitrate scavenging ability (NSA) and antioxidative activities than water single extracted case.

3.5 Polyphenol and DPPH Radicle Activity

There was many technics and methods published and introduced in the textbook for the analyzing methods of polyphenol and DPPH radicle activity, however, few paper published in PBS larvae focused case. Especially, Seo et al. [18] searched that the following contents: they published about the total poly-phenol and DPPH radicle scavenging activity in PBS larvae. The results summerised following: 1) the moisture content was 4.7%, which mostly depends of drying method and system such as air, heat-air, sun, freeze-drying. 2) other nutrients total ash protein, total fat, carbohydrate were 7.4, 18.3, 12.9%, respectively. The protein part, most researchers expressed as total protein and the range was 52-58%. 2) They also analyzed that total polyphenol content and anti-oxidant activity (DPPH radical scavenging activity, ABTS radical scavenging activity, ferric reducing antioxidant power, reducing power) and the total polyphenol contents were 7.7, 6.4, 4.2 MeOH mg GAE/g in 50, 70, and 100% of MeOH, respectively. They used MeOH for analyzing the antioxident activity and that content in 0.3125-10.0 mg/mL, and DPPH radical scavenging activity were very variable range such as 10.4~74.7, 12.1~71.4, 8.8~26.4% in 50%, 70%, 100% MeOH, respectively. The results mean that the increasing of MeOH content increased DPPH radical scavenging activity effectively. Additionally, ABTS radical scavenging activity, ferric reducing antioxidant power, reducing power also increased by the increasing of MeOH. And the 50% MeOH showed high anti-oxidant activity (AOA) compared to other treatments which means AOA dependents on the total polyphenol content.

4. THE ELIMINATION METHODS OF CONSUMERS DISGUST

4.1 The Elimination of Consumers' Disliking Factors

In the past, most PBS larvae was intake from the compost dump of rice straw or oak tree. Additionally it not developed the processing methodology, so most people dislike, especially

lady and young age, it because of the body appearance and compost decaying smell, so historically it consumed by liver disease infected patient only before getting permission as general food from Ministry of Food and Drug Safety [1]. The external appearance and compost smell could be possible by fermentation of PBS larvae instead of direct or simple intake additionally it possible by change of the shape and nutrients together such as larvae's mushroom culture (*Cordyceps militaris*; In Korean which called Dong Chung Hwa Cho and means Winter Insect and Summer Plant). Most researchers tried to overcome or eliminate the disliking reasons and found the best method was perfectly change of the larvae shape with cultivation of mushroom in the larvae body by inoculation of mushroom seeds [4], which technic was basically used in silkworm and the medicinal functionality is very high in the antioxidative and nitrate scavenging activities [24].

One of the many functionality improved production methods, here are three good papers introduced for the industrial insect researchers and consumers.

4.2 Larvae's Characteristics and Effective Human Body Character

The previous paper was published by Lee (2018) and that results are summarized that 1) the properties of Aqueous extract of PBS larvae and mountain Ginseng (Korean language pronounciation, SANSAM- historically the most effective for human health medicinal plant in Korea, China) fermented by *Lactobacillus Brevis* and which results are summarized that Lactobacillus brevis SM61 from traditional Kimchi, which is most basic fermented major groceries made of Chinese cabbage and it consume more than 60 kg per year per person and similar to rice consumption), was used for fermentation of aqueous extract of PBS larva and mountain ginseng. 2) The aqueous extract of PBS larva and fermented mixture of aqueous extract and mountain ginseng did not show specific cellular toxicity in RAW264.7 cells until a concentration of 5-1000 μg/mL. 3) The polyphenol contents was highest in the fermented mixture of aqueous extract and mountain ginseng. 4) DPPH radical scavenging activity was stronger in the fermented mixture of aqueous extract and mountain ginseng than the aqueous extract only. 5) Also, antibacterial activity was tested against E. coli, L. monocytogenes and S. aureus. 6) The fermented mixture of aqueous extract and mountain ginseng showed antibacterial activity against the tested bacteria. 7) Therefore, L. brevis SM61 as a starter might be used to improve functionality of PBS larva.

4.3 Improving the Medicinal Effects and Elimination Disgust from Consummers

In this part, Sim et al. [25] published paper and that contents were summarized as : The optimum fermentation conditions in PBS larvae using Bacillus subtilis and that results are summarized that 1) the biological activities of aqueous extract of PBS larvae fermented by *Bacillus subtilis* KACC 91157. 2) On the other hand, the soluble solids of PB increased reaching the maximum level about 5.6 °Brix after 7 days of fermentation. 3) Protein concentration also increased during the fermentation period and the highest protein content measured in the PB fermented for 6 days with *B. subtilis*. Protein content was analyzed by SDS (sodium dodecyl sulfate)-polyacrylamide gel electrophoresis, and it was observed that most of the protein bands were completely degraded after 3 days of fermentation. The phenolic and flavonoid compounds were higher in the fermented group than the non-fermented group. They showed the highest amounts during 2-3 days of fermentation. The highest reducing power and DPPH (α,α'-diphenyl-β-picrylhydrazyl) radical scavenging activity were observed in PB fermented for 3 days, and no major changes occurred during the subsequent fermentation. Similarly, fibrinolytic activity was the highest at the 3rd day of fermentation. Finally, they concluded that fermentation of PB for 3 days by *B. subtilis* is the optimum time to achieve the desired physicochemical properties and highest biological activities.

5. CONCLUSIONS

1. PBS larvae extraction more functionality than powder type without extraction or fermentation.
2. PBS larvae give positive effects on therapeutic effects, prevention of breast cancer, inflammatory disease, human oxidation, and liver-related diseases.
3. Blood related disease or symptoms might be over-come by intake of PBS larvae.
4. Consumers disliking factors overcoming by fermentation or changing of appearance and funtionality with mushroom cultivation in the body of PBS larvae.

5. Using of combination both fermentation and processing technics can improve the PBS larvae's functionality and which directly resulting good effects for the farmers as economically viable, especially small farm scale and consumers getting good functionality foods after well processed larvae or mushroom without any hesitation.
6. If we eating food insect just some part, many adult diseases could be preventive and cure
7. If we increase consuming of food insect, global warming of our earth could be decreasing

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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