

Quality Evaluation of Hot Air-Dried Edible Insect '*Protaetia Brevitarsis* Larvae' Including Sacrificing Method, Storage Temperature, and Defatting Condition

Ha Young Kim, Duri Kim, and Ji-Yeon Chun*

Department of Food Science and Engineering, Jeju National University

Abstract

This study evaluated the *Protaetia brevitarsis* larvae powder's characteristic changes using hot air drying (60±2.5°C, 12 h) with different pre-treatment methods, including two sacrifice methods, two storage temperatures, and two defatting processes. Appearance, yield, moisture contents, pH, color, proximate analysis, volatile basic nitrogen level, DPPH radical scavenging activity, and total phenol content were assessed. Results revealed that a combination of blanching, defatting, and -20°C storage temperature resulted in higher total phenol contents, lower water contents, and lower volatile basic nitrogen levels than other methods. Defatted treatment resulted in a higher L-value than the non-defatted treatment. Taken together, these results indicate that a combination of -20°C storage, blanching, and defatting is the optimal pre-treatment method for obtaining *P. brevitarsis* larvae powder with high total phenol content, low water content, and low volatile basic nitrogen, taking into account cost efficiency considerations.

Keywords: *Protaetia brevitarsis* larvae, sacrificing method, hot-air drying, defatting, food quality

Introduction

The Food and Agriculture Organization of the United Nations (FAO) has predicted that the population will increase to 10 billion by 2050, which will increase environmental pollution due to food and feed production increases (FAO, 2017). Edible insects have an eco-friendly advantage with fewer environmental pollution problems because they have lower gas emission such as carbon dioxide (CO₂), methane (CH₄), and nitrogen dioxide (N₂O) than conventional livestock such as pigs and cattle known to have high reproductive power during being raised (Van Huis, 2016; Orsi et al., 2019). In addition, protein contents of edible insects range from 31% to 65%, higher than those of beans (23.5% of protein), lentils (26.7%), and soybeans (41.1%). Thus, edible insects are attracting attention as alternative protein sources for the future food (Gould & Wolf, 2018). As of 2021, ten edible insects including *Batryticatus Bombyx*, silkworms (larva, pupa), grasshoppers, *Tenebrio molitor*, *Gryllus bimaculatus*, *Protaetia brevitarsis* (larva), *Allomyrina dichotoma*, *Zophobas morio*, *Apis mellifera*, and *Locusta migratoria* have been recognized

as food ingredients in Korea.

Among these ten edible insects in Korea, *P. brevitarsis* larvae is an insect with complete metamorphosis (eggs, larvae, pupa, and adults) and it belongs to the family of Coleoptera. It is widely distributed in Korea, Japan, Taiwan, China, and Europe (Chung et al., 2013). *P. brevitarsis* larvae contain about 17% carbohydrates, 58% proteins, and 18% fat. It has been reported that they possess various beneficial properties, including high-quality protein, antioxidant activity, anti-thrombotic efficacy, neuroinflammation-inhibitory effect, and liver function improving effect (Kwon et al., 2013; Lee et al., 2017; Choi et al., 2019; Lee et al., 2019).

Edible insects can easily become rancid during storage due to their high protein and lipid contents. Therefore, pre-treatment methods are important. The Belgian Federal Food Safety Authority (FASFC) has stated that edible insects need heat treatment such as blanching to reduce the number of microorganisms (Vandeweyer et al., 2017). Methods of pre-treatment and preservation of edible insects include roasting, frying, blanching, steaming, dipping, smoking, and baking. The blanching method is the most preferred pre-treatment method for various raw food stuff and edible insects. The heat of hot water can penetrate into insects and sacrifice them in a short time to prevent deterioration. It is an appropriate method for reducing microorganisms and foreign substances (Rumpold et al., 2014; Son et al., 2016; Dagostin, 2017). Sacrificing method is certainly a key step in insect processing. However,

Co-first author: Ha Young Kim and Duri Kim.

*Corresponding author: Ji-Yeon Chun, Department of Food Science and Engineering, Jeju National University, Jeju-si 63243, Korea.

Tel: +82-64-754-3615; Fax: +82-64-755-3601

E-mail : chunjiyeon@jejunu.ac.kr

Received February 2, 2022; revised February 26, 2022; accepted March 4, 2022

not enough research has been done and there are no specific regulations regarding the insect processing. (Caligiani et al., 2019; Larouche et al., 2019; Leni et al., 2019). Study on the black soldier fly, highlighted that Sacrificing method of prepupae impact the lipid composition. Furthermore, Larouche et al. (2019) and Singh et al. (2020) reported that sacrificing method has a significant influence on the final quality of insect. Longer sacrifice times induced stress, which is associated with increased metabolism of energy reserves (metabolism of triglycerides to acylglycerol and free fatty acids) and promotes oxidation (Caligiani et al., 2019).

As insects are prone to color change and oxidation of PUFA lipid fractions during processing, challenges remain to be addressed (Caligiani et al., 2019; Leni et al., 2019). There are several studies on methods of sacrificing insect. However, there are few studies assessed the effect of different sacrificing methods, defatting process, and storage method, on the quality of the final product. Base on this, we considered a sacrificing method that is industrially convenient and minimizes stress on insect. Therefore, in this study, live *P. brevitarsis* larvae were processed to produce *P. brevitarsis* larvae powder, by using blanching, freezing, storage temperature, and defatting treatment and investigated their effect on the quality characteristics of *P. brevitarsis* larvae powder.

Materials and Methods

Materials

Folin-Ciocalteu phenol reagent, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), gallic acid and L-ascorbic acid were purchased from Sigma Aldrich (St. Louis, MO, USA). All other chemicals used were of analytical grade.

Preparation of *P. brevitarsis* larvae powder

The third instar larval stage of *P. brevitarsis* larvae were obtained from Jeju insect farming association corporation. *P. brevitarsis* larvae were fasted for 7 days to remove all rubbish and then they were washed before sacrificing. *P. brevitarsis* larvae were sacrificed by blanching (95°C, 1 min) and quick freezing (-80°C). And then they were vacuum packed and stored in a freezer at -80°C or -20°C. Stored *P. brevitarsis* larvae were hot-air dried at 60°C (PS-100C, Shiniltech, Gimhae, Korea) until below 15±5% moisture contents.

Defatting *P. brevitarsis* larvae powder

Dried *P. brevitarsis* larvae were ground to a fine powder using a grinder (SHMF-3500G, Hanil, Seoul, Korea), it was

pass through a 40-mesh sieve. The insect sample was defatted using n-hexane. A 1:5 ratio of hexane (w/v) was mixed with powder and stirred for 18 h. Every 6 hour, after filtration, Fresh hexane was added. Defatted samples were dried under a fume hood to evaporated remained hexane.

Appearance, yield, approximate composition

The appearance of the *P. brevitarsis* larvae on white plate was observed every process. The yield of the defatting and drying was calculated as a weight percentage before and after drying. The proximate composition of the powder was analyzed following the AOAC (1990) procedures.

pH and color measurements

The pH was measured in triplicate by vortexing 1 g of powder in 10 mL of distilled water. The pH was determined digital pH-meter (S 470 SevenExcellence™, Inti Inc., Schwerzenbach, Switzerland). The color was measured in triplicate with a chroma meter (CR-210, Minolta Co., Osaka, Japan). A standard plate (L=91.29 a=1.69 b=-12.86) was used as the standard.

Determination of volatile basic nitrogen (VBN)

Protein deterioration was observed by measuring volatile base nitrogen contents. The official method of the commission of the Korea Food and Drug Administration (Ministry of Food and Drug Safety, 2020) was applied. Briefly, 10 g of samples were stirred in 50 mL distilled water for 30 min and filtered. The samples were neutralized to weak acidity by adding 5% H₂SO₄. After addition of 1 mL of the above samples and 1 mL of saturated potassium carbonate solution to the outer chamber of the conway unit and add 1 mL of 0.01 N H₂SO₄ into the inner section. The lid was closed and incubated at a 25°C for 1 h. Then titrated with 0.01 N NaOH. The VBN content was expressed in mg%.

Total phenolic content

The powder's total phenolic content was determined with the Folin-Ciocalteu method as described (Singleton & Rossi, 1965) with some modification. Briefly, 200 µL of the sample was mixed with 900 µL of distilled water and 100 µL 2 M Folin-Ciocalteu's phenol reagent. The mixtures were kept in a darkness at room temperature for 5 min. Then, a 300 µL of 2% Na₂CO₃ and 500 µL of distilled water were added to the mixture, which was kept in darkness for 1 h. The absorbance at 760 nm of all samples was measured. Gallic acid was used as a standard.

DPPH radical scavenging activity

The antioxidant activity of the powder was measured as described (Mensor et al., 2001) with some modification. Briefly, 2 mL of sample was mixed with 800 μ L of 0.3 mM DPPH (in 95% ethanol). The mixtures were kept in a darkness at room temperature for 30 min. The absorbance at 517 nm of all samples was measured. Ascorbic acid was used as a standard. The radical scavenging activity (%) was calculated by the following formula:

$$\text{DPPH radical scavenging activity (\%)} = \left[1 - \frac{A_1}{A_0}\right] \times 100 \quad (1)$$

A_1 : sample absorbance

A_0 : blank control (i.e., DPPH solution with distilled water) absorbance

Statistical analysis

Minitab 18 (Minitab Inc., State College, PA, USA) was used. All experiments were carried out in triplicate and results were reported as the mean \pm standard deviation. Significant differences ($p < 0.05$) between treatments were determined with One way ANOVA, followed by Tukey and an independent-samples T-test was used to analyze the mean comparison according to defatting treatment.

Results and Discussion

Appearance and Yield

The appearance of *P. brevitarsis* larvae is shown in Fig. 1. In general, these larvae crawl around using their back because their legs are not developed. It was found that each body segment of the larvae had hairs. The growth of *P. brevitarsis* larvae could be divided in first instar, second instar, and third instar larval stages. Sizes of *P. brevitarsis* larvae were 0.3-20 mm, 25-30 mm, and 40-45 mm, respectively during growing. Third instar larvae of *P. brevitarsis* larvae were used in this study. The color of blanched larvae was whiter than that of larvae without the blanching treatment. Insect browning can occur due to enzymatic reactions caused by enzymes such as tyrosinase, tyrosine hydroxylase, L-DOPA decarboxylase, laccase, and peroxidase (Singh et al., 2020) or non-enzymatic reactions in which amino acids and reducing sugars can react with each other to produce melanoidins (David-Birman et al., 2018). It is possible to use blanching method to sacrifice the larva in a short time by applying high heat to the larva. However, a quick-freezing method takes a long time to completely sacrifice insects due to metabolic heat (Cooper et

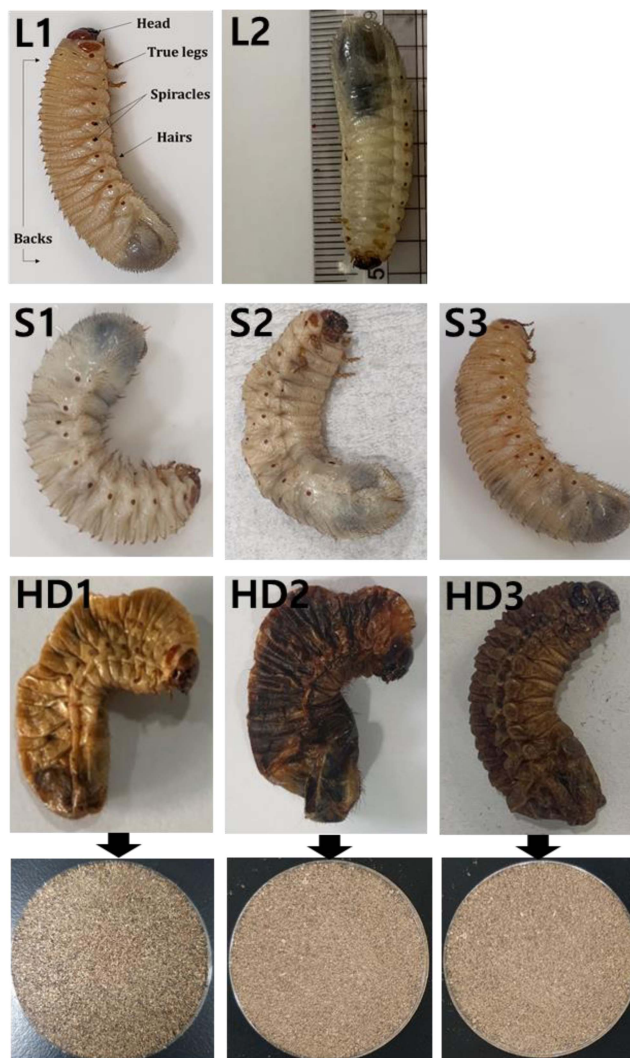


Fig. 1. Appearance of *Protetaia brevitarsis* larvae prepared by different processing methods, including different sacrifice methods, storage temperature. L1 and L2: live and fasted *Protetaia brevitarsis* larvae (about 4 cm), S1: blanched at 95°C and stored at -20°C, S2: blanched at 95°C and stored at -80°C, S3: frozen at -80°C and stored at -80°C, HD1: hot-air dried S1, HD2: hot-air dried S2, HD3: hot-air dried S3.

al., 1984). For this reason, non-heating sacrificial methods such as quick freezing do not inactivate enzymes or microorganisms in the larvae, resulting in a darker color of the final product. According to Leni et al. (2019) black soldier fly (BSF) killed by freezing shows a darker color than blanched BSF. In the present study, blanching was also effective in prevent browning. All larvae pretreated by the three methods (S1: blanched at 95°C and stored at -20°C, S2: blanched at 95°C and stored at -80°C, S3: frozen at -80°C and stored at -80°C) did not maintain their original appearance after hot air drying. They were deformed because of rapid evaporation of moisture.

Defatting yield and drying yield of *P. brevitarsis* larvae powder processed under various conditions are shown Fig. 2. There was no significant ($p>0.05$) difference in the defatting yield of powder obtained by different sacrificial methods and storage temperatures. In the case of protein foods with high fat contents, the defatting process can directly affect protein recovery and purity of the final product. *P. brevitarsis* larvae have high fat contents, so if they are not defatting process, the powder will aggregate. In addition, its yield will decrease, and fat oxidation can occur during storage. Therefore, a defatting process is needed to improve the storage stability of the powder.

Proximate composition

The proximate composition of *P. brevitarsis* larvae powder

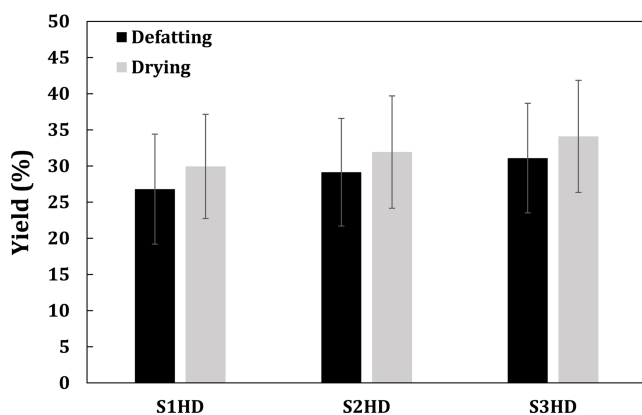


Fig. 2. Defatting and drying yields of *Protaetia brevitarsis* larvae powder prepared with different processing conditions. There was no significant differences in defatting or drying yield among samples ($p>0.05$). S1HD: blanched at 95°C and stored at -20°C and hot-air dried, S2HD: blanched at 95°C and stored at -80°C and hot-air dried, S3HD: frozen at -80°C and stored at -80°C and hot-air dried.

samples obtained with a defatting process and different sacrificial methods are summarized in Table 1. Previous studies on nutrient composition (% Dry Matter Basis) of *P. brevitarsis* larvae have indicated that they have abundant nutrients, including crude protein content ranging from 44.23% to 66.65%, crude fat content ranging from 10.40% to 26.81%, and crude ash content ranging from 4.45% to 8.45% (Yeo et al., 2013; Back et al., 2017; Kim et al., 2020; Lee et al., 2020). In general, neither sacrificial method seemed to have any significant effect on the proximate composition of *P. brevitarsis* larvae powder in the present study. S1HD, S2HD, and S3HD showed moisture contents of 8.59%, 11.80%, and 15.34%, respectively. In this study, we obtained average moisture content and fat contents of 78.07 g/100 g and 3.54 g/100 g fresh weight of *P. brevitarsis* larvae before drying, respectively. After drying and defatting processes, the moisture content was between 8.15% and 15.34% and the fat content was between 0.82% and 1.04%. Lipid oxidation stability of *P. brevitarsis* larvae could be improved by reducing the fat content. There was no significant ($P>0.05$) difference in protein, fat, ash, or carbohydrate content according the processing condition. Defatted powder had protein content of 54.61%-56.81%. This content was much higher than those found for eggs, meat, and fish. High-level proteins might improve the functionality of the powder (Kim et al., 2021). In this study, *P. brevitarsis* larvae showed high protein contents independently from the sacrificial method. This suggests that *P. brevitarsis* larvae are highly valuable as a food ingredient. The WHO/FAO has explained that a food can be called protein-rich if its protein content is 10 g/100 g edible part (Udomsil et al., 2019).

The crude ash content of defatted *P. brevitarsis* larvae was 5.09% to 6.34%, which was similar to or lower than results of

Table 1. pH and color of *Protaetia brevitarsis* larvae powder obtained with different processing conditions such as different sacrificing methods, storage temperatures.

Defatting	S1HD		S2HD		S3HD	
	Non-defatting	defatting	Non-defatting	defatting	Non-defatting	defatting
pH	7.17±0.04 ^B	7.12±0.05 ^a	7.31±0.15 ^A	7.32±0.14 ^a	6.51±0.08 ^C	7.09±0.45 ^{a*}
L* ¹⁾	44.31±1.61 ^A	60.05±5.81 ^{a*}	44.64±0.42 ^A	60.75±2.30 ^{a*}	41.67±0.33 ^B	59.45±0.93 ^{a*}
a*	1.10±0.21 ^A	2.06±0.79 ^{a*}	2.22±1.02 ^A	2.64±1.14 ^a	1.30±1.28 ^A	2.21±1.55 ^a
b*	1.81±1.09 ^B	4.51±0.91 ^{a*}	4.00±1.32 ^A	5.80±1.86 ^a	3.02±1.34 ^{AB}	5.21±2.33 ^a

All values are presented as mean ± SD

¹⁾L*: lightness, a*: redness, b*: yellowness

^{A-C} Means with the same letter are significantly different ($p>0.05$)

^a Means with different letter are not significantly different ($p<0.05$)

S1HD: blanched at 95°C and stored at -20°C and hot-air dried

S2HD: blanched at 95°C and stored at -80°C and hot-air dried

S3HD: frozen at -80°C and stored at -80°C and hot-air dried

other studies (5.72% to 12.09%) (Kim et al., 2020; Kim et al., 2021). However, Ghosh (2017) has reported that five edible insects have higher mineral contents than conventional foods of animal origin, and *P. brevitarsis* larvae having the highest iron contents among minerals.

pH and Color

Results of pH and color of *P. brevitarsis* larvae powder processed under various conditions are shown Table 2. The pH of animal food is an important indicator for a change in its properties during storage. The pH values of *P. brevitarsis* larvae powder obtained with different sacrificial methods and a defatting process were found to be between 6.51 and 7.32. The pH of the S3HD sample was significantly ($p<0.05$) lower than that of S1HD of S2HD before defatting. This might be due to glycolysis. Sanchez-Paz et al. (2006) have shown that insects, like invertebrates, can keep energy reserves in the form of glycogen. Some previous studies using stressed (exhaustion) invertebrates in the field of culinary have shown low pH values (Ellington, 1983; Hervant et al., 1999). This phenomenon is due to accumulation of lactic acid because of glycolysis. In this study, S1HD and S2HD were killed immediately by hot-water blanching. However, S3HD took a relatively longer time to die under -80°C . Thus, *P. brevitarsis* larvae might have been stressed and glycolysis might have occurred during the freezing process. Farina (2017) has reported that the pH of cooked broth made from live insects is lower than that made from frozen insects, and it is consistent with the findings of the present study. The S3HD showed a significantly higher pH after a defatting process. This was generated that the increase in pH is correlated with mineral

Table 2. Proximate analysis of defatted *Protetia brevitarsis* larvae powder with different sacrificing methods and storage temperatures

Components	S1HD	S2HD	S3HD
Moisture	8.59±1.41 ^{b1)}	11.80±1.61 ^{ab}	15.34±1.07 ^a
Crude protein	56.81±4.12 ^a	56.74±1.51 ^a	54.61±2.75 ^a
Crude fat	1.04±0.04 ^a	0.82±0.62 ^a	0.92±0.13 ^a
Crude ash	6.34±0.69 ^a	5.65±0.13 ^a	6.09±0.86 ^a
Crude carbohydrate ²⁾	27.23±4.89 ^a	25.00±3.60 ^a	23.05±3.10 ^a

All values are presented as mean ± SD

¹⁾Different superscripts within rows of the sacrifice method and storage temperature represent significant difference at $P<0.05$ by Turkey's multiple range test

²⁾Crude carbohydrate = 100-(moisture + crude protein + crude fat + crude ash)

^{a-b} Means with the same letter are significantly different ($P>0.05$)
 S1HD: blanched at 95°C and stored at -20°C and hot-air dried
 S2HD: blanched at 95°C and stored at -80°C and hot-air dried
 S3HD: frozen at -80°C and stored at -80°C and hot-air dried

content. Kim et al. (2019) also reported that contents of minerals such as potassium (K), phosphorus (P), and magnesium (Mg) were higher in *Zophobas atratus* larvae after defatting than those before defatting. The pH of insect powder might change depend on the sacrificial method; however, it showed no significant difference according to the sacrificial method due to an increase in mineral content after the defatting process.

The lightness of *P. brevitarsis* larvae powder after defatting was higher ($p<0.05$) than that before defatting, consistent with results of Son et al. (2019). They observed that the lightness value of defatted *Tenebrio molitor* with n-hexane was the highest. Mishyna et al. (2019) have also reported that defatted insect powder has a higher lightness due to fat removal. Kan et al. (2008) have analyzed pigment produced by arthropods and found that phenol oxidase can promote the production of quinone and form melanin pigment. Thus, the lightness might have been increased by eluting the melanin pigment of insects during the defatting process. And the lightness improvement has a positive influence on the consumer preference. Meanwhile, there was no significant ($P>0.05$) difference in the redness value according to the type of Sacrificing method before or after defatting. Dye produced by the arthropods were that the phenol oxidizing enzyme (Phenol oxidase) that promotes the formation of the quinone (Quinone) to form a melanin pigment.

Volatile basic nitrogen (VBN)

The VBN value is an important indicator to evaluate the freshness of an animal product. The VBN is a generic term of volatile, basic nitrogen-containing substances such as ammonia, primary amines, and secondary amines produced through decomposition of proteins by enzymatic reactions and bacteria. The quality of insects with high protein contents can be influenced by protein deterioration. According to the Korean Food Standards Codex, the VBN in fresh meat should be under 20 mg%. The VBN of *P. brevitarsis* larvae powder according to the sacrificial method and defatting process in the present study was below 20 mg%. In the case of insects, it is difficult to apply the standards for fresh meat. However, this was carried out to confirm the tendency of protein deterioration according to pretreatment. Results of VBN of *P. brevitarsis* larvae processed under various conditions are shown in Fig. 3. After defatting, there was a significant ($p<0.05$) difference in VBN of *P. brevitarsis* larvae powder according to the pre-treatment method. Due to the difference in moisture content of the raw material according to the

sacrificial method, S3HD took 15 h to dry, while S1HD and S2HD only took 12 h to dry. For this reason, a higher decomposition of protein was expected for *P. brevitarsis* larvae powder obtained with freezing as a sacrificial method. In addition, the VBN of S2HD was higher than that of S1HD probably due to a higher moisture in S2HD. Proteins are associated with lipids in food structures and membranes. Thus, protein co-oxidation is strongly interconnected with lipid oxidation in protein products. Indeed, protein could react with most intermediate and final products from lipid oxidation. The lower the moisture, the slower the rate of co-oxidation (Schaich, 2013; Estevez, 2011; Tournour et al., 2017). Conversely, Singh et al. (2020) have observed that thermal treatment such as blanching can lead to the highest protein and lipid oxidation, which is also increased with longer storage period. Quick freezing method resulted in a lower initial oxidation of the

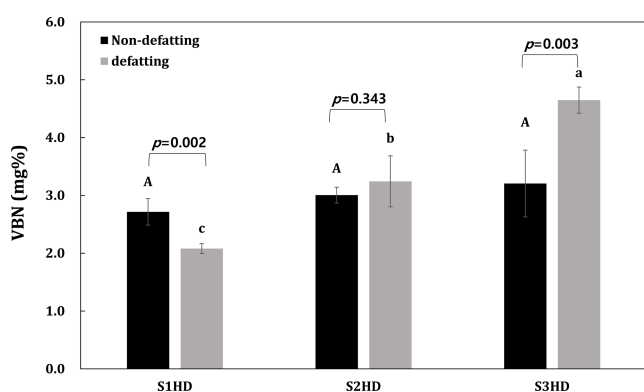


Fig. 3. Volatile basic nitrogen (mg%) of *Protactia brevitarsis* larvae powder after different processing methods such as different sacrificing methods, storage temperatures. Error bar indicates standard error. Results of before and after treatment of each sample were compared using the two-sample *t*-test ($p < 0.05$). ^AMeans with the same letter are not significantly different ($p > 0.05$). ^{a-c}Means with different letter are significantly different ($p < 0.05$). S1HD: blanched at 95°C and stored at -20°C and hot-air dried, S2HD: blanched at 95°C and stored at -80°C and hot-air dried, S3HD: frozen at -80°C and stored at -80°C and hot-air dried.

powder which did not increase with storage period. The difference trend from this study might be due to different conditions of blanching used in this study.

In addition, between before and after defatting of each sample, results were compared using two-sample *t*-test ($p < 0.05$). Results of the experiment showed that the defatting process affected protein deterioration in S1HD and S3HD. The protein deterioration of *P. brevitarsis* larvae powder might be due to differences in components after defatting. Food proteins are sensitive to oxidative reactions, leading to possible degradation of food properties such as lower digestibility (Estévez, 2011). Results of the present study suggest that defatted S1HD exhibits lower VBN value, thus having a potential for food applications.

Total phenolic contents and DPPH radical scavenging activity

Total phenol content is commonly used to indicate antioxidant levels. Phenolic compounds are secondary metabolites present in the plant world. They can be used as an indirect indicator of antioxidant activity. Total phenolic contents of *P. brevitarsis* larvae processed under various conditions are shown Table 3. Total phenol content of *P. brevitarsis* larvae sacrificed by freezing at -80°C was higher ($p < 0.05$) than that by blanching. Polyphenol component of *P. brevitarsis* larvae was destroyed by blanching heat. Defatted *P. brevitarsis* larvae powder showed higher phenol content than the non-defatted powder.

The protein content of defatted *P. brevitarsis* larva powder was higher than that of non-defatted larvae powder. Thus, it would be easy for defatted powder containing high protein content to bind to polyphenols. Suh & Kang (2011) have indicated that the feeding trait is responsible for phytochemical compounds in *P. brevitarsis* larvae. In the present study, *P. brevitarsis* sacrificed by freezing at -80°C showed the highest total phenolic content. Further study is needed to determine the

Table 3. DPPH radical scavenging activities and total phenolic contents of *Protactia brevitarsis* larvae powder obtained with different processing conditions such as different sacrificing methods, storage temperatures.

Defatting	S1HD		S2HD		S3HD	
	Non-defatting	Defatting	Non-defatting	Defatting	Non-defatting	Defatting
Total phenolic contents (mg GAE/g)	4.42±0.28 ^B	5.04±0.36 [*]	4.20±0.12 ^B	5.15±0.13 ^{b*}	4.78±0.18 ^A	5.58±0.26 ^{a*}
DPPH free radical scavenging (%)	53.67±2.51 ^A	52.17±2.53 ^a	47.86±5.51 ^A	55.05±4.49 ^a	52.37±4.81 ^A	52.83±5.37 ^a

All values are presented as mean ± SD

^{A-B} Means with the same letter are significantly different ($p > 0.05$)

^{a-b} Means with different letter are significantly different ($p < 0.05$)

S1HD: blanched at 95°C and stored at -20°C and hot-air dried

S2HD: blanched at 95°C and stored at -80°C and hot-air dried

S3HD: frozen at -80°C and stored at -80°C and hot-air dried

association between protein level and polyphenol component.

To measure the antioxidant activity, DPPH radical scavenging activity assay is a widely utilized. Results of DPPH radical scavenging activity of larvae powder samples obtained with various pretreatment conditions are shown Table 3. There was no significant difference ($p>0.05$) in DPPH radical scavenging activity (47.86-55.05%) among samples. All powder samples after a defatting process showed antioxidant activities. Most of them showed the DPPH radical scavenging activity of 52.17% to 55.05% ($p>0.05$). Singh et al. (2020) have also reported that DPPH radical scavenging activities of crickets sacrificed by the blanching method and the freezing method at -20°C followed by hot air drying are 60.4% and 59.6%, respectively, showing no significant ($p>0.05$) difference between the two. Therefore, the type of sacrificial method and defatting process did not affect the DPPH radical scavenging activity.

From all results of this study, it indicated that S1HD (blanching, defatting, -20°C storage) was an optimal processing method for producing *Protaetia brevitarsis* larvae powder. Blanching can secure food safety by inactivating enzymes and preventing protein deterioration as much as possible by reducing drying time in terms of quality. The combination of blanching and a defatting process can improve the storage stability by removing lipid. A defatting process is an essential process for extending the shelf life and securing the food safety of edible insects rich in lipids. At -20°C storage would be more economical by preventing protein deterioration, although low temperature (-80°C) effectively maintained antioxidant activity components of insect powder. Moreover, it was suitable to have more proteins in dried food.

Conclusion

Effects of various pre-treatment methods (blanching, quick-freezing, storage temperature, defatting treatment) on characteristics of *Protaetia brevitarsis* larvae were investigated. Results of this study indicated that S1HD (blanching, defatting, -20°C storage) was an optimal processing method for producing *Protaetia brevitarsis* larvae powder. Blanching can secure food safety by inactivating enzymes and preventing protein deterioration as much as possible by reducing drying time in terms of quality. Moreover, a low temperature of -80°C is suitable to maintain antioxidant activity components. However, to have more proteins in dried food, the combination of blanching and -20°C storage would be more economical by preventing protein deterioration. A defatting process can improve the storage stability by removing lipid. A defatting

process is an important process for extending the shelf life and securing the food safety of edible insects rich in lipids.

Acknowledgments

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. 2019R1F1A1062721).

Author Disclosure Statement

The authors declare no conflict of interest.

References

- AOAC. 1990. Official Methods of Analysis. Association of Official Analytical Chemists (No. 934.06), Washington, DC, USA.
- Back MH, Hwang JS, Kim MA, Kim SH, Goo TW, Yun EY. 2017. Comparative analysis of nutritional components of edible insects registered as novel foods. *J. Life Sci.* 27:334-338.
- Caligiani A., Marseglia A., Sorci A., Bonzanini F., Lolli V., Maistrello L., Stefano S. 2019. Influence of the killing method of the black soldier fly on its lipid composition. *Food Res. Int.* 116:276-282.
- Choi IH, Yu R, Lim YJ, Choi GS, Choi SU, Hwang JI, Son JS, Chung TH. 2019. Antithrombotic efficacy of *Protaetia brevitarsis*. *J. Environ. Sci. Int.* 28: 639-643.
- Chung MY, Hwan JS, Goo TW, Yu EY. 2013. Analysis of general composition and harmful material of *Protaetia brevitarsis*. *J. Life Sci.* 23: 664-668.
- Cooper JE, Ewbank R, Rosenberg ME. 1984. Euthanasia of tortoises. *Vet. Rec.* 114: 635.
- Dagostin JLA. 2017. Use of blanching to reduce antinutrients, pesticides, and microorganisms. Springer, Berlin, Germany. P. 61-94.
- David-Birman T, Raften G, Lesmes U. Effects of thermal treatments on the colloidal properties, antioxidant capacity and *in-vitro* proteolytic degradation of cricket flour. *Food Hydrocoll.* 79: 48-54.
- Ellington WR. The recovery from anaerobic metabolism in invertebrates. *J. Exp. Zool.* 1983. 228: 431-444.
- Estévez M. 2011. Protein carbonyls in meat systems: A review. *Meat Sci.* 89: 259-279.
- Farina MF. 2017. How method of killing crickets impact the sensory qualities and physicochemical properties when prepared in a broth. *Int. J. Gastron. Food Sci.* 8: 19-23.
- Food and Agriculture Organization of the United Nations. The

- future of food and agriculture trends and challenges. Available from: <http://www.fao.org/3/a-i6583e.pdf>. Accessed Aug. 20. 2020.
- Ghosh S, Lee SM, Jung C, Meyer-Rochow VB. 2017. Nutritional composition of five commercial edible insects in South Korea. *J. Asia Pac. Entomol.* 20: 686-694.
- Gould J, Wolf B. 2018. Interfacial and emulsifying properties of mealworm protein at the oil/water interface. *Food. Hydrocoll.* 77: 57-65.
- Hervant F, Garin D, Mathieu J, Freminet A. 1999. L-lactate metabolism and glucose turnover in the subterranean crustacean niphargus virei during post-hypoxia recovery. *J. Exp. Biol.* 202: 579-592.
- Kan H, Kim CH, Kwon HM, Park JW, Roh KB, Lee H, Park BJ, Zhang R, Zhang J, Söderhäll K, Ha NC, Lee BL. 2008. Molecular control of phenoloxidase-induced melanin synthesis in an insect. *J. Biol. Chem.* 283: 25316-25323.
- Kim TK, Yong HI, Chun HH, Lee MA, Kim YB, Choi YS. 2020. Changes of amino acid composition and protein technical functionality of edible insects by extracting steps. *J. Asia Pac. Entomol.* 23: 298-305.
- Kim TK, Yong HI, Kim YB, Jung S, Kim HW, Choi YS. 2021. Effects of organic solvent on functional properties of defatted proteins extracted from *Protaetia brevitarsis* larvae. *Food Chem.* 336: 127679.
- Kim YS, Kim GH, Ko HJ, Kim MA, Kim IW, Seo MC, Lee JH, Lee HJ, Baek MH, Hwang JS, Yoon HJ. 2019. Comparative analysis of nutrients and hazardous substances in *Zophobas atratus* Larvae. *J. Life Sci.* 29: 1378-1385.
- Kwon EY, Yoo JM, Yoon YI, Hwang JS, Goo TW, Ki MA. 2013. Pre-treatment of the white-spotted flower chafer (*Protaetia brevitarsis*) as an Ingredient for novel foods. *J. Korean Soc. Food Sci. Nutr.* 42: 397-402.
- Larouche J, Deschamps MH, Saucier L, Lebeuf Y, Doyeon A, Vandenberg GW. 2019. Effects of killing methods on lipid oxidation, colour and microbial load of black soldier fly (*Hermetia illucens*) larvae. *J. Anim. Sci.* 9: 182.
- Lee HJ, Seo MC, Lee JH, Kim IW, Kim SY, Hwang JS, Kim MA. 2019. Inhibitory effect of *Protaetia brevitarsis seulensis* ethanol extract on neuroinflammation in LPS-stimulated BV-2 microglia. *J. Life Sci.* 29: 1096-1103.
- Lee HS, Ryu HJ, Song HJ, Lee SO. 2017. Enzymatic preparation and antioxidant activities of protein hydrolysates from *Protaetia brevitarsis* larvae. *J. Korean Soc. Food Sci. Nutr.* 46: 1164-1170.
- Lee SM, Choi YS, Jo Kyung, Kim TK, Yong HI, Jung S. 2020. Quality characteristics and protein digestibility of *Protaetia brevitarsis* larvae. *J. Anim. Sci.* 62:741-752.
- Leni G, Caligiani A, Sforza S. 2019. Killing method affects the browning and the quality of the protein fraction of Black Soldier Fly (*Hermetia illucens*) prepupae: a metabolomics and proteomic insight. *Food Res. Int.* 115: 116-125.
- Mensor LL, Menezes FS, Leitão, GG, Reis AS, Santos TCD, Coube CS, Leitão SG. 2001. Screening of brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytother. Res.* 15:127-130.
- Ministry of Food and Drug Safety (MFDS). Korea Food Coed. Available from: http://www.foodsafetykorea.go.kr/foodcode/01_03.jsp?idx=11142. Accessed Mar. 24. 2020.
- Mishyna M, Martinez JI, Chen J, Benjamin O. 2019. Extraction, characterization and functional properties of soluble proteins from edible grasshopper (*Schistocerca gregaria*) and honey bee (*Apis mellifera*). *Food Res. Int.* 116: 697-706.
- Orsi L, Voegelé LL, Stranieri S. 2019. Eating edible insects as sustainable food? Exploring the determinants of consumer acceptance in Germany. *Food Res. Int.* 125: 108573.
- Rumpold BA, Fröhling A, Antje Fröhling, Reineke K, Knorr D, Boguslawski S. 2014. Comparison of volumetric and surface decontamination techniques for innovative processing of mealworm larvae (*Tenebrio molitor*). *Innov. Food Sci. Emerg. Technol.* 26: 232-241.
- Sánchez-Paz A, García-Carreño F, Muhlia-Almazán A, Peregrino-Uriarte AB, Hernández-López J, Yepiz-Plascencia G. 2006. Usage of energy reserves in crustaceans during starvation: status and future directions. *Insect Biochem. Mol. Biol.* 36: 241-249.
- Schaich KM. 2013. Lipid Oxidant. Logan. Elsevier Applied Science, London, UK, p. 1-52.
- Singh Y, Cullere M, Kovitvadhi A, Chundang P, Dalle Zotte A. 2020. Effect of different killing methods on physicochemical traits, nutritional characteristics, in vitro human digestibility and oxidative stability during storage of the house cricket (*Acheta domesticus* L.). *Innov. Food Sci. Emerg. Technol.* 65: 102444.
- Sinleton, VL, Rossi JA. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* 16: 144-158.
- Son YJ, Lee JC, Hwang IG, Nho CW, Kim SH. 2019. Physicochemical properties of mealworm (*Tenebrio molitor*) powders manufactured by different industrial processes. *LWT-Food Sci. Technol.* 116: 108514.
- Son YJ, Ahn W, Kim SH, Park HN, Choi SY, Lee DG. 2016. Study on the oxidative and microbial stabilities of four edible insects during cold storage after sacrificing with blanching methods. *J. Korean Soc. Food Sci. Nutr.* 29: 849-859.
- Suh HJ, Kang SC. 2011. Antioxidant activity of aqueous methanol extracts of *Protaetia brevitarsis* Lewis (Coleoptera: Scarabaedia) at different growth stages. *Nat. Prod. Res.* 26: 510-517.
- Tournour, HH, Segundo, MA, Magalhães, LM, Costa, AS,

- Cunha, LM. 2017. Effect of Touriga nacional grape extract on characteristics of mechanically deboned chicken meat kept under frozen storage. *J. Food Process Eng.* 40: e12434.
- Udomsil N, Imsoonthornruksa S, Gosalawit C, Ketudat-Cairns. 2019. Nutritional values and functional properties of house cricket (*Acheta domesticus*) and field cricket (*Gryllus bimaculatus*). *Food Sci. Technol.* 25: 597-605.
- Van Huis A. 2016. Edible insects are the future. *Proc Nutr Soc.* 75: 294-305.
- Vandeweyer D, Lenaerts S, Callens A, Campenhout LV. 2017. Effect of blanching followed by refrigerated storage or industrial microwave drying on the microbial load of yellow mealworm larvae (*Tenebrio molitor*). *Food Control* 71: 311-314.
- Yeo HL, Youn KJ, Kim MJ, Yun EY, Hwang JS, Jeong WS, Jun MR. 2013. Fatty acid composition and volatile constituents of *Protaetia brevitarsis* Larvae. *Prev. Nutr. Food Sci.* 18: 150-156.
- Yoon CH, Jeon SH, Ha YJ, Kim SW, Bang WY, Bang KH, Gal SW, Kim IS, Cho YS. 2020. Functional chemical components in *Protaetia brevitarsis* Larvae: Impact of supplementary feeds. *Food Sci. Anim. Resour.* 40: 461-473.

Author Information

Ha Young Kim: Graduate Student, Jeju National University

Duri Kim: Graduate Student, Jeju National University

Ji-Yeon Chun: Professor, Jeju National University