Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.7517

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **7 (3):** 503-509 (2019)

Research Article

International Journal of Pure & Applied Bioscience

Extraction and Characterization of Silkworm Pupae (*Bombyx mori*) Oil by LC-MS/MS Method

Akshay R. Patil, Gagan Dip, R. Meenatchi*, Jeyan Arthur Moses and S. Bhuvana

Indian Institute of Food Processing Technology, Thanjavur, Tamilnadu, India

*Corresponding Author E-mail: meena@iifpt.edu.in

Received: 12.04.2019 | Revised: 17.05.2019 | Accepted: 23.05.2019

ABSTRACT

Entomophagy is a new trending terminology where insects are used as source of food and feed. There are 1900 species of insects reported as edible, where in that silkworm pupae (Bombyx mori) is one of them. Studies were undergone using silkworm pupae where it was dried and powdered subjected to proximate analysis. Oil was extracted using soxhlet extraction method where the oil is further studied for its quality parameters and for fatty acid profile using LC-MS/MS. Proximate results showed that silkworm pupae with of 42.53% protein and 34.86% fat. Pupae oil quality parameters revealed that acid value (2.63 mg KOH/g), saponification value (182.43 mg KOH/g), iodine value (117 g iodine/100g of oil), peroxide value (2.66 meq/kg) from the silkworm pupae. The fatty acid profile of oil screened around 28 fatty acids among which 12.75% were saturated and 86.6% were unsaturated fatty acids. MUFA and PUFA were around 34.97% and 51.64% respectively. The main component in pupae oil with essential fatty acid of 28.35% contains both omega-3 and omega-6 fatty acids which are more important for curing cardiovascular diseases and diabetes. Silkworm pupae oil is a potential edible oil can be used for replacement of other edible oils for better health benefits.

Key words: *Edible insects, Silkworm pupae oil, mono unsaturated fatty acids, poly unsaturated fatty acids, essential fatty acids.*

INTRODUCTION

Food is essential for everyone's life; Current status shows that by 2050 the world population will be around 9 billion, around 821 million undernourished people will be in the whole world, Food insecurity can occur to solve this problem, for that insects can be alternative food for whole population. Already many studies carried out for insect as a food and feed for the future world. Traditionally edible insects are consumed by around 2 billion people. Till now around 1900 insect's species are reported that can be used as food. Insects are alternative sustainable protein source for humans and animals. It has estimated that at least 113 countries consuming around 2000 species of insect species[9].

Cite this article: Patil, A.R., Gagan Dip, Meenatchi, R., Moses, J.A., Bhuvana, S., Extraction and Characterization of Silkworm Pupae (*Bombyx mori*) Oil by LC-MS/MS Method, *Int. J. Pure App. Biosci.* **7(3):** 503-509 (2019). doi: http://dx.doi.org/10.18782/2320-7051.7517

Commonly consumed insects are flies (Diptera 2%), dragonflies (Odonata: 3%), termites (Isoptera: 3%), leafhoppers, plant hoppers, scale insects and true bugs (Hemiptera: 10%), crickets, locusts, grasshoppers (Orthoptera: 13%), ants, bees, wasps (Hymenoptera: 14%), caterpillars (Lepidoptera: 18%), beetles (Coleoptera: 31%) and others (5%)[15]. *B. mori* is the insect comes under Lepidoptera order, where it is using commercially used for producing silk yarn. India is the second largest producer of silk which contributes 31,906 MT in year 2017 as the data given by International Sericulture Commission, United Nations. India is the only country in the world which produce all four verities like Mulberry, Eri, Tasar and Muga. Largest practiced sericulture industry is mulberry silkworm which contributes 76% of the entire silkworm production and 90% in world production.

Mulberry leaves are fed for growing of larvae to produce silk by forming cocoon and are known as mulberry silk. Consumption of silkworm pupae is traditionally practiced in South Korea, China, Malaysia and some parts of European countries. In China, consumption of silkworm pupae approved as novel food source by Ministry of Health of the People Republic of China[17]. In India, consumption of silkworm pupae is practising by tribal from early days in north east part of India. Silkworm pupae (*Bombyx mori*) having very significant role in nutritional composition. As silkworm pupae contributes 60% of the cocoon weight where it was discarded as waste material after use. Silkworm can be used improve the health benefits of the society. Silkworm pupae oil is considered as a good source of edible oil which can be used in some various applications like food, medicine and cosmetics The objective of the study is to analyse proximate composition of silkworm pupae and its fatty acid profile for the benefit of mankind.

MATERIAL AND METHODS

2.1 Sample collection and preparation

Silkworm cocoon samples were collected from the Ariyanapatti village farm Thanjavur. After 5 to 6 days the pupae is fully developed in cocoon, then pupae are taken out from cocoon balls by cutting. Then the sample screened with defected ones, then went for washing thoroughly to remove foreign materials. The pupae sample were dried in hot air woven for 60-70^{o}C for overnight and then dried samples were collected and grounded to powder form by using the mixer for further analysis[10]. The dried powder of insect's subject to defatting using N-hexane to get defatted sample which will enhance the protein content of the powder[3].

2.2 Sample nutritional analysis

Insects are having its own importance in nutritional profile where Silkworm pupae (*Bombyx mori*). The proximate compositions were done according to the Association of Official Analytical Chemists international methods[1].

2.2.1 Proximate analysis

Moisture content, ash, fat, crude fibre of dried silkworm pupae powder was determined using methods 934.01, 942.05, 920.39, 962.09 respectively. Then Protein content was determined by using nitrogen conversion factor (6.25 x N) from AOAC kjeldahl method (984.13)[10].

2.3 Pupae Oil extraction

Dried silkworm pupae (*Bombyx mori*) powder is loaded in a cellulose thimble closed with a good cotton and kept into a chamber of soxhlet extractor for 4-6 hour and petroleum ether used as a solvent and then after finishing the process oil was separated by evaporating a solvent which is present in oil[14]. Then the oil is stored in glass bottle for the further analysis.

2.4 Oil quality parameter analysis

The extracted pupae oil was characterized for their quality parameters viz., saponification value, iodine value, peroxide value and acid value (free fatty acid content).

2.4.1 Acid value (free fatty acid content)

Acid value was determined where 1-10 g of oil was dissolved in ethanol and ether (1:2, v/v) with alcoholic KOH (potassium hydroxide) then added 100µL of 1% phenolphthalein as an indicator[8].

2.4.2 Saponification value

Saponification value of pupae oil was estimated by adding 4-5 g of oil sample with 50 ml of alcoholic KOH then dissolved and connected the flask to air condenser, gently boiled about 1hr. Oil was cooled and condensed then add 1 ml of 1% phenolphthalein indictor was added and titrated against 0.5N HCl until the pink colour disappears and do same for blank.

$$SV = \frac{28.05 \times \ (titrate\ value\ of\ blank - titrate\ value\ of\ sample)}{weight\ of\ the\ sample(g)} \times 100$$

2.4.3 Peroxide value

For peroxide values estimation 2-4 g of oil was dissolved in 25ml of acetic acid and chloroform in the ratio of 3:2 by the volume, then 1 ml of saturated potassium iodide solution was added and kept in dark for 10 min. Add 1% starch solution and 30 ml distilled water and the mixture was titrated with 0.01N sodium thiosulphate until the blue colour gets disappeared[8].

2.4.4 Iodine value

For estimation of iodine 0.3g of oil dissolved in 10ml of chloroform and 25ml of Wijs iodine solution was added. The solution was kept in dark for 30 min, then the it was mixed with 20ml of 20% potassium iodide solution and 100 ml distilled water. It was titrated against 0.1N sodium thiosulphate and 1% starch as an indicator[8].

2.5 Fatty acid profile analysis by LC MS/MS

An approximately 100 mg of pupa oil and two millilitres of sodium methoxide (CH_3ONa), (0.5 *M*) in Me OH (14%, wt/ vol) was added. The tubes were incubated in a 55°C water bath for 1.5 h with vigorous hand-shaking for 5 s every 20 min. Then 2 ml of a NaHCO3 a saturated solution and 3 ml of hexane were added to it. The tubes were vortex to mix properly. After centrifugation, the hexane layer containing the FAME was placed into a LC-MS vial. The vial was capped and placed at −20°C until LC-MS analysis. The sample was evaporated using nitrogen and it was re dissolved with 5 ml of LC–MS grade methanol. The sample was purified using Solid Phase Extraction (SPE) cartridge for RP-LC/ESI-MS/MS analysis[7].

RESULTS AND DISCUSSION

3.1 Proximate analysis of silkworm pupae:

Physio chemical properties of silkworm pupae is presented in Table.1, where the proximate were the mean value of the protein was around 42.53% dry basis and the lipid content was around 34.86%. The mean value of ash and moisture showing 4.79 and 4.83%, respectively. The crude fibre content was 0.056% which is in negligible amount. The proximate of silkworm pupae powder showed that it is rich in nutrients which can be comparable to past studies[11].

Table 1: Proximate analysis of silkworm pupae powder (per 100g)

Nutrients	Silkworm pupae (*Bombyx mori*)
Protein (g)	42.53±1.05
Fat (g)	34.86±0.83
Crude Fibre (g)	0.056±0.015
Ash (g)	4.796±0.015
Moisture (db)%	4.83±1.08

Silkworm pupae powder contain more protein shown around 42.53% which can be comparable to our regular food like meat (20-25%), milk (7-10%), legumes (15-20%), egg (7%), fish (30%) so that can be replace to other protein rich foods. Silkworm pupae is also containing more fat around 35%) which comparable to sunflower oil (41%), safflower oil (35%), sesame seed oil (50%), coconut dry (68%), groundnut (56%)[4]. where the silkworm pupae were a useful insect which have good nutritive value which will be a better replacement for above foods.

3.2 Analysis of pupae oil quality:

Fatty acid which is defined as the weight of KOH in mg needed to neutralize the organic acids present in 1 g of fat where the silkworm having 2.3% of free fatty acid value which can be comparable with the perilla and camellia oils which is reported by Ze-Yuan Deng *et.al.*[2]. Saponification value represents the amount of alkali require to saponify the 1g of oil[12] where the pupae oil gives around 182.43 mg/g which can be near to safflower seed and palm oils which is having 190.23 and 205 mg/g respectively[5]. Iodine value of pupae oil was 117g/100g which comparable to canola oil which is having around 110-120 g/100g[13]. Peroxide value is will gives the initial evidence of rancidity in unsaturated fats and oils, where in pupae oil having 2.66 meq/kg which is comparable to palm oil which is having 2.2 meq/kg. This silkworm pupae oil having good quality parameters which can be used as edible oil and other purposes also.

Table 2: Quality evaluation of silkworm pupae oil

Parameters	Result
Free fatty acid (mg KOH/g)	2.63±0.20
Saponification value (mg KOH/g)	182.43±1.05
Iodine value (g iodine/100g of oil)	117±1.55
Peroxide value(meq/kg)	2.66±0.03

3.3 Fatty acid profile analysis:

Fatty acid profile analysis was carried using LC-MS/MS. Fig.1 represents the full spectrum of fatty acid profile of oil has the 28 fatty acids screened. It has unsaturated fatty acids including both Mono Unsaturated Fatty Acid (MUFA) and Poly Unsaturated Fatty Acid (PUFA). Saturated fatty acid is in negligible amount 12.75% in oil and unsaturated fatty acids were high 86.61%. Fig. 2 represents the total ion chromatogram of pupae oil.

The saturated fatty acids present in silkworm pupae oil shown in Table. 3. Around 10 fatty acids were detected in oil where it is having 0.20% Undecyclic acid (C11:0), 0.11% Lauric acid (C12:0), 3.80% Myristic acid (C14:0), 1.32% Pentadecylic acid (C15:0), 0.74% Palmitic acid (C16:0), 1.28% Stearic acid (C18:0), 2.73% Archidic acid (C20:0) relative peak area percentage, where total 12.75% of saturated fatty acids represents the pupae oil, in that myristic acid, arachidic acid, behenic acid, palmitic acid were more which is not good for human diet which can cause risk of cardiovascular diseases.

Table 3: Saturated fatty acids in silkworm pupae oil (*Bombyx mori*)

Formula	Common name	Mass to charge (m/z)	Relative Peak area %
$C_{11}H_{22}O_2$	Undecylic Acid	185	0.201831
$C_{12}H_{24}O_2$	Lauric Acid	199	0.111601
$C_{13}H_{26}O_2$	Tridecylic Acid	213	0.076271
$C_{14}H_{28}O_2$	Myristic Acid	227	3.802968
$C_{15}H_{30}O_2$	Pentadecylic Acid	241	1.328889
$C_{16}H_{32}O_2$	Palmitic Acid	255	0.742853
$C_{18}H_{36}O_2$	Stearic Acid	283	1.288006
$C_{20}H_{40}O_2$	Arachidic Acid	311	2.730986
$C_{22}H_{44}O_2$	Behenic Acid	339	2.475748

The total unsaturated fatty acids present in silkworm pupae oil is around 86.61%. In that having both monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) which is around 34.97% and 51.64% respectively of relative peak area. In the

MUFA, oleic acid (C18:0), Erucic acid (C22:1), Nervonic acid (C24:1) and 11-Eicosenoic acid (C20:1) with good amount which will against cardiovascular disease. It provides more membrane fluidity than saturated fatty acids, where consumption of more monounsaturated fatty acids and decreasing in saturated fatty acids intake will improve the insulin sensitivity. Around 28.35% and 12.75 % essential and nonessential fatty acids were present in pupae oil, where as in PUFA which all are essential fatty acids containing omega-3 (ω-3) and omega-6 (ω-6) fatty acids which are having more than one double bond which is not synthesize in our body. In the silkworm pupae oil the main component is gamma linolenic acid (C18:3), Linolenic acid (C18:3), Linoleic acid (C18:2) Arachidonic acid (C20:4), Homogamma Linolenic acid (C20:3), Eicosatrienoic acid (C20:3), Eicosadienoic acid (20:2), Arachidonic acid with more peak area percentage of 34% and all PUFA which recognised as a good fat. Omega-3 fatty acids which is present in algal oil, fish oil, fish which the results have shown the lower risks of myocardial infraction. These omega-6 fatty acids which is present in safflower oil and sunflower oil may reduce the risk of cardiovascular disease[16]. Docosahexaenoic acid (DHA) is an omega-3 fatty acid which is a structural component of human brain, retina, skin and cerebral cortex which is given in the name of 20:6 (n-3) can be synthesised from alpha linolenic acid (ALA) or can be obtained from maternal milk directly and in some oil like fish oil and algae oil[6]. Most of the beneficial fatty acid as mentioned above were present in silkworm pupae oil.

Table 4: Unsaturated fatty acids in Silkworm pupae oil (*Bombyx mori*)

Formula	Common name	Mass to charge (m/z)	Relative Peak area %
$C_{14}H_{26}O_2$	Physeteric acid	225	0.936505
$C_{16}H_{30}O_2$	Palmitoleic acid	253	0.680885
$C_{17}H_{32}O_2$	Heptadecenoic Acid	267	1.019474
$C_{18}H_{30}O_2$	Gamma Linolenic Acid	277	1.193393
$C_{18}H_{30}O_2$	Linolenic Acid	277	1.331277
$C_{18}H_{30}O_2$	Linoleic Acid	279	4.778698
$C_{18}H_{34}O_2$	Oleic Acid	281	3.672072
$C_{18}H_{34}O_2$	Elaidic Acid	281	3.718576
$C_{20}H_{32}O_2$	Arachidonic Acid	303	33.95754
$C_{20}H_{34}O_2$	Homogamma Linolenic Acid	305	1.057048
$C_{20}H_{34}O_2$	Eicosatrienoic Acid	305	1.064677
$C_{20}H_{36}O_2$	Eicosadienoic Acid	307	2.536302
$C_{20}H_{38}O_2$	Eicosenoic Acid	309	4.530636
$C_{20}H_{38}O_2$	Eicosenoic Acid	309	4.518909
$C_{22}H_{32}O_2$	Docosahexaenoic Acid	327	3.95522
$C_{22}H_{40}O_2$	Docosadienoic Acid	335	1.763992
$C_{22}H_3O_2$	Erucic Acid	337	8.191849
$C_{24}H_{46}O_2$	Nervonic Acid	365	7.705268

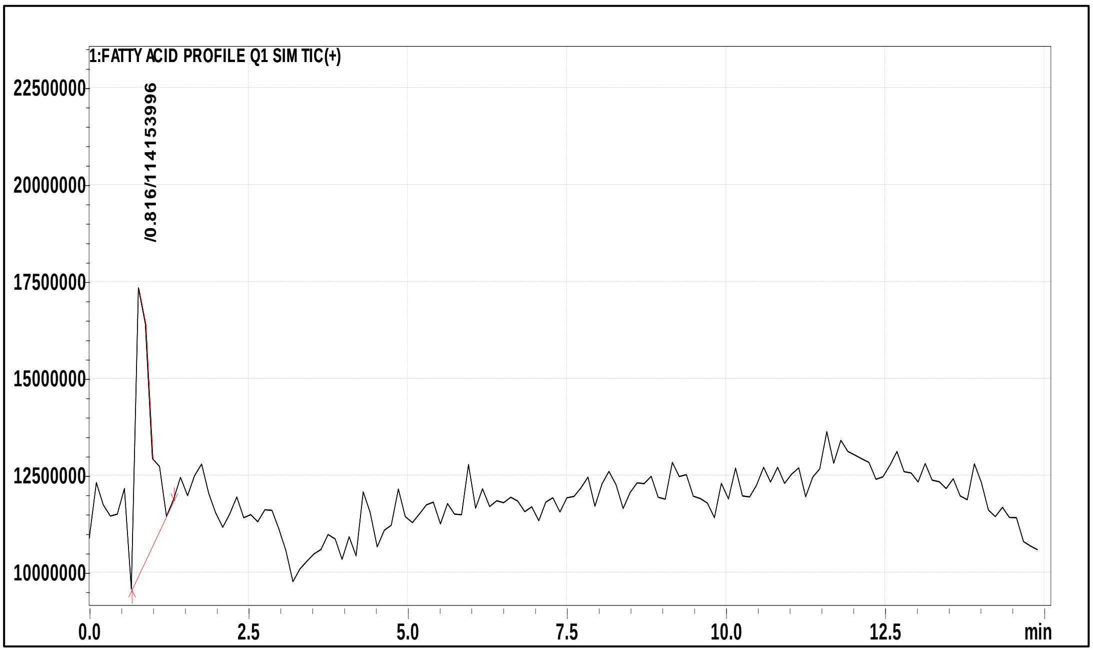

Fig. 1: LC-MS/MS Full spectrum of fatty acid profile

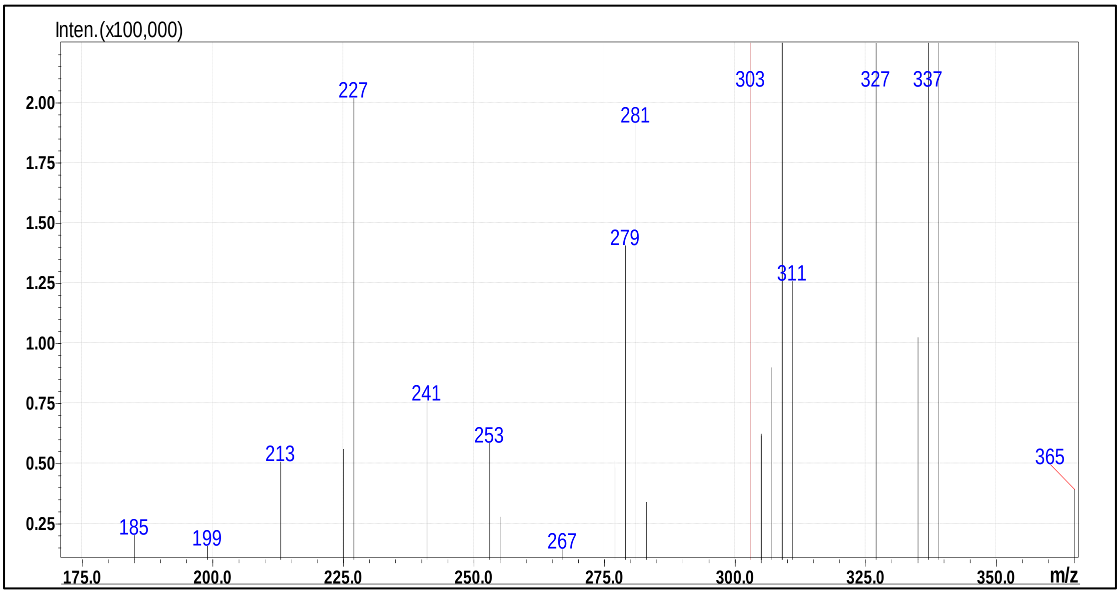

Fig. 2: LC-MS/ MS Total Ion Chromatogram (TIC) of Pupae Oil Sample

CONCLUSION

Silkworm pupae (*Bombyx mori*) is very beneficial has more protein and fat. The present study shows that pupae oil with good quality fatty acid profile, especially MUFA and PUFA which is beneficial for health. Essential fatty acids such as omega-3 and omega-6 which are present in pupae oil is comparable to fish oil and algae oil and higher than other vegetable oils. Docosahexaenoic acid (DHA) an omega-3 fatty acid necessary for organ development of premature babies, it is found naturally in maternal milk and some of the sea foods. The same DHA found in silkworm pupae oil which can be an effective supplement for human nutrition. Hence the pupae oil can be used as a replacement for other edible oils and can be future food with healthy fats which can prevent the risk of many diseases.

REFERENCES

1. AOAC, Official methods of analysis, 18th ed. [Revised] Association of Official

Analytical Chemists. Washington, DC (2006).

2. Cao, J., Li, H., Xia, X., Zou, X. G., Li, J., Zhu, X. M., & Deng, Z. Y., Effect of fatty acid and tocopherol on oxidative stability of vegetable oils with limited air. *International journal of food properties*, **18(4):** 808-820 (2015).
3. Choi, B. D., Wong, N. A., & Auh, J. H., Defatting and Sonication Enhances Protein Extraction from Edible Insects. *Korean journal for food science of animal resources*, **37(6):** 955 (2017).
4. Ebrahem, K. S., Richardson, D. G., Tetley, R. M., & Mehlenbacher, S. A., Oil content, fatty acid composition, and vitamin e concentration of 17 hazelnut varieties, compared to other types of nuts and oil seeds. In *III International Congress on Hazelnut* 351 (pp. 685-692) (1992, September).
5. Gopinath, A., Puhan, S., & Nagarajan, G., Theoretical modeling of iodine value and saponification value of biodiesel fuels from their fatty acid composition. *Renewable Energy*, **34(7):** 1806-1811 (2009).
6. Guesnet, P., & Alessandri, J. M., Docosahexaenoic acid (DHA) and the developing central nervous system (CNS)–implications for dietary recommendations. *Biochimie*, **93(1):** 7-12 (2011).
7. O’Fallon, J. V., Busboom, J. R., Nelson, M. L. and Gaskins, C. T., A direct method for fatty acid methyl ester synthesis: Application to wet meat tissues, oils, and feedstuffs. *J Anim Sci* **85:** 1511-1521 (2007).
8. Jeon, Y. H., Son, Y. J., Kim, S. H., Yun, E. Y., Kang, H. J., & Hwang, I. K., Physicochemical properties and oxidative stabilities of mealworm (Tenebrio molitor) oils under different roasting conditions. *Food science and biotechnology*, **25(1):** 105-110 (2016).
9. Jongema, Y., List of edible insects of the world. Wageningen University & Research, Wageningen, the Netherlands (2017).
10. Longvah, T., Mangthya, K., & Ramulu, P., Nutrient composition and protein quality evaluation of eri silkworm (Samia ricinii) prepupae and pupae. *Food Chemistry*, **128(2):** 400-403 (2011).
11. Pereira, N. R., Ferrarese-Filho, O., Matsushita, M., & de Souza, N. E., Proximate composition and fatty acid profile of Bombyx mori L. chrysalis toast. *Journal of Food Composition and Analysis*, **16(4):** 451-457 (2003).
12. Predojević, Z. J., The production of biodiesel from waste frying oils: A comparison of different purification steps. *Fuel*, **87(17-18):** 3522-3528 (2008).
13. Seneviratne, Kapila & Jayathilaka, Nimanthi, Coconut Oil: Chemistry and Nutrition (2016).
14. Thirupathaiah, Y., Sivaprasad, V., Bhuvaneswari, E., Reddy, M. M., & Chandrashakharaiah, M., Extaction and Characterization of mulberry silkworm, BOMBYX MORI L., pupae oil. *Indian Journal of Sericulture*, **55(1-2):** 31-37 (2016).
15. Van Huis, A., Van Itterbeeck, J., Klunder, H., Mertens, E., Halloran, A., Muir, G., Vantomme, P., Edible Insects. Future Prospects for Food and Feed Security. FAO: Rome, 201 p (2013).
16. Willett, W. C., The role of dietary n-6 fatty acids in the prevention of cardiovascular disease. *Journal of Cardiovascular Medicine*, **8:** S42-S45 (2007).
17. Zhou, J., & Han, D., Safety evaluation of protein of silkworm (Antheraea pernyi) pupae. *Food and chemical toxicology*, **44(7):** 1123-1130 (2006).

