Available online at www.easletters.com

ISSN No: 2349-2864

Entomology and Applied Science Letters, 2016, 3, 5:182-188

Housefly Larvae as a Source of Good Quality Renewable Protein Product

Ruzalia Ulanova and Irina Kravchenko*

Winogradsky Institute of Microbiology,Research Center of Biotechnology of the Russian Academy of Sciences, Moscow, Russia

** Corresponding Email: irinakravchenko@inbox.ru*

ABSTRACT

The preparation and characterization of protein product from housefly larvae with lye solution are reported. The results indicated that, extraction temperature, sodium hydroxide concentration, and extraction time had significant effect on the yield and the quality of the extracts. The optimal preparation conditions were solution-material ratio of 5:1, extraction temperature of 80°C, extraction time of 20 min, and sodium hydroxide concentration of 0.2% (w/v). When prepared under optimal conditions, yield of housefly larvae proteins was 57.53%, and its sensory, physical and chemical properties and sanitary index were found to be of high edible level and hygiene value andwell-balanced in essential and non-essential amino acids ratio.We concluded that a new preparation procedure is nontoxic and inexpensive, making this a perspective organic process.

Keywords: Domestic fly, Protein extraction method, Insect-based products

INTRODUCTION

The Earth's population is expected to exceed well over 9 billion by 2050, and there is an urgent need to increase the supply of protein from sustainable sources. Insects are now seriously considered as an alternative and additional source of protein in developed countries and insect-based proteins provide an alternative for protein sources derived from conventional livestock[1, 2].Insect meals are rich in proteins and lipids [3] and could represent a good source of nutrients in animal production and aquaculture [4]. Insects do not use metabolic energy to maintain a constant body temperature and canõtherefore invest more energy in growth. Farming of insects (mini-livestock)requires less water and space than the production of conventional livestock, and the insects are growing on the side streams from the agro-industry [5]. Research and business activities around the topic of insects for food and feed have increased over the last years in view of the fast growing body of scientific literature and the global interest in this topic. The prognosis of a future move towards high protein diets in combination with a projected lack for agriculturally suitable land expansion, has led to a renewed focus on the use of insects as potential protein sources in the human food chain [6].

There remains a wide gap between activities being conducted, largely for food in developing countries and the high-tech, large-scale industrial initiatives primarily in developed countries. Some insect species (black soldier fly and housefly, mealworms, crickets, and grasshoppers)are currently produced, for example, by Agri Protein, Enviro Flight, Enterra, Hermetia, Proti-Farm, Altamed companies, in industrial quantities and used as feed for particular fish species, poultry, pigs and pets. Until now there are only a few companies in Russia which produce live fly larva for poultry and fishing (Fauna Centre, Eco Baits, Silver Fish, NovyeTechnologii, etc). To realize the potential of insects, assuring a sustainable supply of the insect protein resourceis required. This can be achieved by further investigation of insect processing and biofractionation in relation to properties relevant for food and feed industry.

The significant part of plant protein produced on croplands is fed to food animals. Over half of this fed protein is lost to waste and is unavailable in the animal products. Additionally, one fourth of all the food produced is lost to waste during the distribution and consumption of the food supply chain. The housefly (*Muscadomestica*) is currently the object of considerable, worldwide interest as a prospective candidate for recovering protein and energy from

waste streams. It has several desirable characteristics for this purpose: communal feeding habit, non-pest status, efficient digestion, high protein and lipid content, and low incidence of disease and other mortality factors [7]. Industrial-scale, insect farms producing house fly larvae could be an important source of protein for feeds and lipid for biofuels [8]. Fly larvae have an amazing ability to convert fresh manure to compost in a very short time into a sustainable and resource-efficient alternative protein as well as food and feed source. From 1000 kg of manure (30 % dry matter) about 70 kg larvae is produced and the crude protein content varies between 40 and 60 %, and lipid content ranged between 9 to 25% of dry matter. It can be utilized for the conversion and valorization of organic waste and has a high potential for the application e.g. in the aquaculture as a fish meal replacer [9]. House fly larvae (maggots) are widely applied as a natural component of protein and lipids in the diet of fish, chicken, and pig. Plant and animal agricultural byproducts derived after extraction of a high- value component can provide lower cost sources, which is particularly important for animal feed.

The great potential of insects as an alternative protein source becomes apparent when comparing their protein content with that of the plants such as soybeans or animal by-products such as meat. Insects would be qualified as "protein concentrates" with protein content ranging from 30 percent in wood worms to 82 percent in some species of wasps. Their digestibility values go from 33 percent up to 96 percent in some moth and butterfly larvae [10]. Protein quality and nutritional value are determined by the amino acid composition and the digestibility of the protein fraction. The 20 proteinogenic amino acids are classified as indispensable or dispensable and nine of them histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine)are classified as essential as they cannot be synthesized in the human body. .The important parameter of the protein quality is the ratio between essential (E) and nonessential (N) amino acids. According to FAO/WHO criteria, E/(E + N) has to reach about 40% with E/N = 0.6 [11].Insects can fulfill some human nutritional requirements and most of them contain the high-value protein sources with an essential amino acid score (percentage in an ideal protein) ranges from 46% to 96% [12]. Similarly, the amino acid composition of insect meals differs largely among species. High amino acid values for phenylalanine and tyrosine have been found in some species, and some insects are rich in tryptophan, lysine and threonine [13, 14].

The housefly (*Muscadomestica Linnaeus* 1758) is the most common fly (Diptera) species. Due to the ability of housefly maggots to grow on a large range of substrates they are very useful in transformation of wastes into a valuable biomass rich in protein, fat, and chitin. The fly larvae are very nutritious and are value natural food source for poultry, aquaculture and ruminant nutrition [15, 16].Currently, there are a lot of successful examples of the production of housefly maggot biomass in controlled conditions for poultry or in pond farming. They have an amino acid composition that is similar to fishmeal, and which is especially rich in the essential amino acids [17]. Different authors have reported different nutritional values for maggot meal attributed to variations in species, age, method of processing and substrate [18]. Recently the development of active antibacterial substances from fly maggots as a replacement in animal feed has become an active area of research [19, 20].

Although in the recent years intensive efforts have been made to study intact edible insects, still very little information from a food science point of view is available on characteristics and functionality of extracted insect proteins. The aim of this study is to extract proteins from fly larvae in order to characterize the obtained protein fractions and to evaluate their nutritional properties in the context of food and feed application. To the best of our knowledge, no studies initiatives are focused on this subject matter and no information are available. The specific objectives of this study were: (a) to elaborate the procedure of insect protein extraction and characterize protein fraction based on protein quality and stability; (b) to study protein content and quality by amino acid analysis.

MATERIALS AND METHODS

Sample preparation

Live housefly *Muscadomestica* larvae (maggots) were kindly provided by the commercial insect farm `Fauna Centre`, Moscow Region, Russia. Mature maggots were harvested from a pool of maggot grown in a fish wastes substrate on the 3rd day of larvae formation. Harvested samples were sieved and stored alive at 4ºC for about one day before processing. Fresh insects were frozen at -80^{o}C and then freeze-dried to determine moisture and dry matter content gravimetrically.

Extraction of crude protein

25 g portion of fresh larvae was rinsed and boiled in distilled water for 15 min and subsequently blending for one minute (Braun Multiquick 5, Germany)in . Then the insect suspension was sieved through a stainless steel filter sieve with a pore size of 500 µm and the suspension and the residues were collected. After centrifugation at 15 000 g for 30 min at 4ºC, three fractions were obtained: the supernatant (protein product), the pellet (solid residues, including chitin), and the fat fraction (lipids).

In order to optimize procedure and to obtain the highest amount and quality of the housefly protein product (HFPP), the one-factor experiments were designed. Four factors (pH, temperature, solid/liquid ratio and time) were varied at three levels for each factor, as shown in Table 1.

Table 1. Factors and levels for design of the experiments

Factor	1	2	3
NaOH concentration, %	0.1	0.2	0.3
Temperature, ^{o}C	50	70	80
Solid/liquid ratio (w/v)	1:5	1:10	1:20
Reaction time, min	10	20	30

Characterization of protein features

Some physical and chemical properties of the HFPP were tested , including color, flavor, viscosity protein, fat, dry matter. Nitrogen content was evaluated using the Kjeldahl procedure. Crude protein content was calculated by the following equation:

Crude Protein (%) = Nitrogen (%) ×6.25.

Microbiological detections were done by the conventional methods. Aerobic mesophilicmicro organisms were enumerated by a total viable plate count using PCA agar (Merck) incubated at 30^{o}C for 48 h. *Enterobacteriaceae* were enumerated in pour-plates of violet red glucose VRBG medium (OXOID) incubated at 37^{o}C for 24 h. Coliforms were enumerated by the multiple-tube fermentation method based on examination of gas formation in a brilliant green lactose broth within 48 h at 35^{o}C. Pathogens, including *Staphylococcus aureus* and *Salmonella*, were screened on trypticase soy broth(Sigma-Aldrich) and Tetrathionate Brilliant Green broth (Difco) according to according to the manufacturer's protocol. Bacterial endospores were determined by giving the heat shock treatment (10 min at 80^{o}C), followed by plating.

Amino acid composition was evaluated in the HFPP samples (30 mg of vacuum dried protein product) hydrolyzed with 10 mL of 6 mol/L HCl. The amino acids were quantified using a Biotronic LC 6000 amino acid analyzer (Biotronic, Germany) and amino acid composition was reported as mg amino acid per g of protein.

Statistical analysis

In this study, each experiment was conducted in triplicate, and the values were expressed as mean ± std. To evaluate the statistical significance of the differences the one-way analysis of variance(ANOVA) was performed.

RESULTS AND DISCUSSION

Protein content of housefly larvae

Our data have demonstrated that housefly maggot from fish wastes substrate harvested on day 3 contained the crude protein of 45.4% at a dry matter level of 92.7%, which may be a proof of the high protein value of this HFP product. We have compared our data with published results and it was noted that the literature values for the crude protein content of house fly larvae ranged between 20.3 % [21] and 67.9 % [15]. Our result is similar to the findings [22, 23], who reported a crude protein content of 47.1-47.4%. The diversity in crude protein content probably is due to differences in either the analysis procedures or the substrate used to produce the maggots.

Optimization of the conditions for HFPP preparation

Many factors affect the extractability of proteins and it is important to recover as much protein as possible during extraction in order to maximize yield. All currently available and relevant methods for preparation of insect protein are based on physical insect disruption, chemical extraction, and enzymatic conversion. Many methods for fractionating insect's products are conceivable [24, 25] but we were motivated to develop simple, fast and inexpensive method combines physical disruption with solvent-free chemical extraction procedure. The principal scheme in presented on Fig.1. There are some important differences in our protocol as compared with other widely used methods for extraction of insect proteins. Firstly, usually, fresh insects are frozen in liquid nitrogen or dried before blending. We propose the killing of fresh larvae by boiling water followed by blending. The application of fresh larvae may increase the yield of the protein product because the drying procedure could lead to the formation of volatile nitrogen compounds and give lower protein values [26].

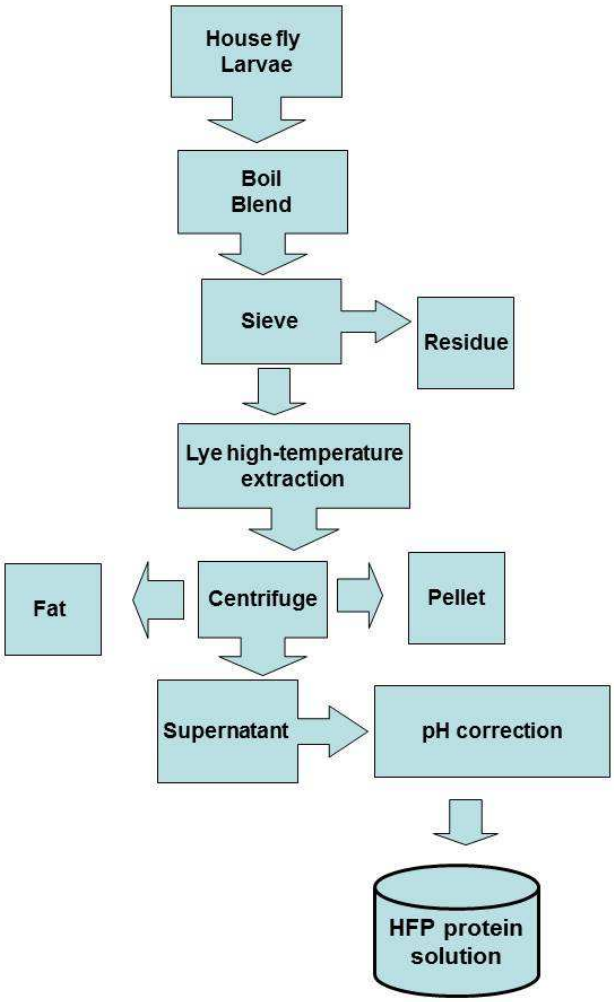

Fig 1.Diagram schematically illustrating the insect protein extraction procedure

At the first step of the procedure biomass was placed into cloth bags, which were immersed in the tank with boiled water for 10-30 min. The specific feature of housefly larvae is rapid blackening of the biomass during storage and processing in aerobic conditions. The chemistry and the nature of this black pigment formation are unclear, but it is possible that complex of melanin with chitin and protein is destroyed during treatment of the larvae. Thus the impact of high temperatures on larvae not only inhibits the formation of black pigment but also disinfects biomass, preventing the ingress of undesirable microorganisms in the target protein product.

Larvae are cultivated on wastes and secondary products of food and agricultural production, which are characterized by a high degree of contamination by microorganisms. Overall levels of 10^8 of a total bacterial count, as far as 10^5 of *Enterobacteriaceae* and 10^4 bacterial spores were found in the of fresh housefly larvae and this is typical for soil and similar materials. Blanching the insects in boiling water has eliminated most of the present *Enterobacteriaceae*, but bacterial spore-forming species are not completely inactivated, and thus could cause risks if favorable conditions return for their germination and growth.

We have investigated the effects of factors levels on physical and chemical properties of the protein product. It was found, that reaction temperature, reaction time and alkalinity were the key factors affecting the features of HFP such as protein content, color, texture and stability. 80^{o}C were preferable, and at 50^{o}C and 70^{o}C crude protein content was lower (data not presented). The 20 min was found to be the optimal extraction time and both decrease to 10 min and increase to 30 min intervals resulted in lower emulsion stability and shelf-life. It was very important to apply 0.2% of NaOH because the product extracted at 0.1% has insufficient characteristics of shelf-life, and increase to 0.3% resulted in darkest (light brown) color among all insect supernatant solutions. These results suggest that optimal

conditions for HFP preparation are 1: 5 ratio of biomass and 0.2% NaOH solution, a reaction temperature 80^{o}C, and a reaction time of 20 min.

Sensory properties are important in determining the use and acceptance of products. Table 2 shows results of the analysis of protein which was produced under the above given optimal preparation conditions. Color has an important influence on the presentation value of a product. HFP solution was white of light-cream color, which may be attractive for the consumer. Our protein products had weak fishy aromas and this could be due to the presence of volatile amines. Flavor depends on the environment where insects live and the feed that they eat and we have studied larvae grown on the fish wastes.

Table 2.Some properties and sanitary indices of the protein product

Sensory properties	
Color	Whiteorlight-cream
Flavor	No unfavorable flavor, weak fish off-aroma
Physic-chemical properties	
Protein, %	6.8± 1.3*
Essential amino acids, % in protein,	51.8± 0.3*
pH	5.71 ± 0.01
Total titratable acidity, ml NaOH 0.1M 10 ml^{-1}	4.4 ± 0.3
Lipids, %	3.9± 1.1*
Microbiological properties	
Total viable aerobic bacterial count (CFU ml^{-1})	15±3*
Enterobacteriaceae	n.d. **
Bacterial endospores (ml^{-1})	18±5*
Yeasts and molds	n.d.
Sanitary indices	
Coliforms	n.d.
Staphylococcus aureus	n.d.
Salmonella	n.d.

**Data are the mean of three replicates ± standard deviation*
***n.d. – Not detected*

The detection and enumeration of indicator organisms are of primary importance for monitoring the sanitary and microbiological quality of protein product. Microbiological parameters, such as viable aerobic counts, *Enterobacteriaceae*, bacterial endospores, and yeasts and molds, are widely used as a measure of the hygienic conditions or quality of food products. Culture-dependent microbial counts showed that the procedure of insect protein extraction is resulted in substance with good microbiological quality. The presence of certain microorganisms indicates the consumption safety of the protein product. The total count of aerobic bacteria and bacterial endospores was 10^2 CFU ml$^{-1,}$ however (Table 2), this level is not a health risk. The results of microbiological analyses were negative for *Enterobacteriaceae*, yeasts and molds. No any pathogenic bacteria or coliforms were found in protein product *(*Table 2).The zero content of pathogenic bacteria and the low presence of mesophilic microorganisms, gives a high hygiene value of HFP protein product.

Amino acid composition and protein quality

Protein quality, and thus nutritional value, is determined by amino acid composition. Amino acids are traditionally classified as nutritionally essential (EAA), “nonessential” (NEAA) or conditionally essential (CEAA), and recently the concept of functional amino acids (FAAs) has been proposed [27]. FAAs are those which participate and regulate key metabolic pathways and improve health, survival, growth, development, lactation, and reproduction of the organisms. Our results showed that HFP product contained17 amino acids including 9 essential amino acids (Table 3). Essential amino acids are those that cannot be synthesized in the body and must be present in the diet. Nine amino acids are classified as EAA (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine). Among EAA lysine and threonine are strictly indispensable since they are not transaminated and their deamination is irreversible. In contrast, the remaining amino acids can participate in transamination reactions. The insect protein quality was estimated by the amino acid composition contained all the essential amino acids in quantities that are necessary for humans [26]. Only eight EAA were found in HFP, and tryptophan and cysteine contents were lower than detection limit. These findings are correlated with data for other insects [24, 26]. The valuable parameter that needs to be considered for the assessment of protein quality is the ratio between essential (E) and nonessential (N) amino acids. According to FAO/WHO criteria, E/ (E + N) has to reach about 40% with E/N = 0.6[28], and in HFP product they were 46.4% and 0.86, correspondently.

Table 3. Amino acid profileof HFP product extracted from housefly larvae grown onfish wages substrate

Amino acid	Unit, mg/g crude protein
Essential amino acid (E)	
Histidine	58.6
Isoleucine	46.4
Leucine	57.9
Lysine	61.8
Methionine	42.6
Phenylalanine	89.4
Threonine	42.6
Valine	64.3
Sum of EAA	463.6
Non-essential amino acid (N)	
Alanine	42.8
Arginine	29.8
Aspartic acid	79.3
Glutamic acid	122.5
Glycine	36.4
Proline	90.3
Serine	35.5
Tyrosine	99.9
E/N	0.86
E/(E+N)×100, %	46.4%

As indicated in Table 4 HFP can be characterized as high value protein source for nutritional requirements. The amount of total essential amino acids was comparable to that of casein and was higher than in other protein rich animal and plant food products. It was observed that tryptophan was not identified in HFP product, while content of the methionine, one of the most limiting essential amino acids, was higher than in any other protein feedstuff including milk casein and fish (Table 4). The content of the other limiting AA lysine was comparable to that in meat and fish, but slightly lower than that of casein. Antagonism between amino acids generally arises in animals from an excess of leucine over isoleucine and valine, the ratio of leucine and isoleucine often is implicated in amino acid antagonism [29]. It was calculated as 1.25 in HFP, that was slightly lower than those of casein, soya and fish (1.50, 1.58 and 1.49, correspondently). The absence of tryptophan may require combination of HFP with another protein source that is high in tryptophan. The reason for the non-identification of tryptophan could be that it was destroyed by hydrolysis procedure. It was reported [30] that the hydrolysis destroys or chemically modifies the asparagine, glutamine and tryptophan residues in protein. Asparagine and glutamine are converted to their corresponding acids (aspartic and glutamic acids), tryptophan is completely destroyed.

Table 4.Comparison of amino acid composition in HFP and some common protein feed stuffs

EAA, % of protein	HFP	Casein*	Soya bean**	Meat***	Fish**
Lysine	6.18	7.4	2.62	5.99	4.55
Histidine	5.86	2.8	1.02	3.96	1.36
Threonine	4.26	4.4	1.66	3.47	2.60
Valine	6.43	6.5	2.06	6.41	3.09
Methionine	4.26	2.5	0.52	0.91	1.68
Isoleucine	4.64	5.5	2.07	0.90	2.97
Leucine	5.79	8.3	3.29	10.1	4.45
Phenylalanine	9.94	4.5	2.12	5.47	2.35
Tryptophan	n.d.	1.1	0.65	1.02	
Cystine	n.d.	0.3	0.74	1.31	
Sum EAA	47.38	43.3	16.75	39.53	23.05

*adapted from * [31]; ** [17]; ***[32].*

CONCLUSION

Currently the attention for insects as sustainable source of protein for humans and livestock is increasing. The nutritional value of insects has been widely recognized, and recently a variety of papers of the insects as food was published [7, 17,33]. There are numerous adventures of the insects as protein source: (a) sustainable source

(environment, economy);(b) animal-based (essential amino acids)and nutritious (high protein content, fatty acids, minerals and vitamins).

Muscadomestica(common house fly) larvae were shown to be a good quality renewable protein source. The simple, easy and feasible to apply method of protein extraction from larvae was created, and some properties of protein product were investigated. The amino acid profile of HFP indicated that it could provide well-balanced essential and non-essential amino acids and could be considered as a good quality protein.

REFERENCES

[1] A.VanHuis.*Annu RevEntomol*, **2013**,58(1), 563-583
[2] L. Kourimska L., A. Adamkova. *NFS Journal*, **2016**, 4, 22–26
[3]M.D.Finke.*Zoo Biology*, **2002**, 21 (3), 269-285.
[4]F.G.Barroso, C. de Haro, M-J. Sanchez-Muros, E.Venegas, A.Martínez-Sánchez, et al. *Aquaculture*, **2014**, 422-423, 193-201
[5] A.T. Dossey, J. A. Morales-Ramos, M. G.Rojas (eds)Insects as sustainable food ingredients**: p**roduction, processing and food applications, Elsevier Inc, **2016**, 402p.
[6]C. L. R. Payne, P. Scarborough, M. Rayner, K. Nonaka. *EJCN*, **2016**, 70**,** 285–291.
[7] A. Stamer. *EMBO reports*, **2015**, 16 (6), 676-680.
[8] H.Wang, Z. Zhang, G.F. Czapar, M.K. Winkler, J. Zheng.*Waste Management Research*, **2013**, 31, 223-231.
[9] E.K. Ajani, L.C. Nwanna, B.O. Musa.*World Aquaculture*, **2004**, 35, 52–54.
[10] F.R. Del Valle, M.H. Mena, H. Bourges.*J Food Process Pres*, **1982**,6(2), 99-110.
[11] FAO. **1989**. Protein quality evaluation. Food and nutrition paper, 51.
[12]S.Belluco, C. Losasso, M. Maggioletti, C. C. Alonzi, M.G. Paoletti, A. Ricci. *Compr Rev Food Sci Food Saf* ,**2013**, 12,296-331.
[13]J. Ramos-Elorduy, J.M. Pino, E.E. Prado,M.A.Perez, J.L.Otero, O.L. de Guevara.
J Food Compos Anal, **1997**, 10(2),142–57.
[14] T. Longvah, K. Mangthya, P. Ramulu.*Food Chem*, **2011**. 128(2), 400–403.
[15] J. Hwangbo, E.C. Hong , A. Jang , H.K. Kang , J.S. Oh , B.W. Kim , B.S.Park*J Environ Biol*, **2009**, 30(4), 609-614.
[16] D.C. Sheppard, J.K. Tomberlin, J.A. Joyce, B.C. Kiser, S.M. Sumner. *J Med Entomol*, **2002**, 39 (4), 695-698
[17] P.S. H.Makkar, G.Tran , V. Heuze, P.Ankers. *Anim Feed Sci Tech*, **2014**, 197, 1-33.
[18] A. B.Rumpold, O.K. Schluter. *MolNutr Food Res*, **2013**, 57, 802–823.
[19]A.-K. Poppel, H. Vogel, J. Wiesner. *Antimicrob. Agents Chemother***, 2015**, **59**(5),**2508**-2514
[20] C. Beemelmanns, H. Guo , M. Rischer, M. Poulsen. *Beilstein J Org Chem*, **2016**, 12, 314–327.
[21] J.O. Ogunji, W. Kloas, M. Wirth, W. Schulz, B. Rennert. *Asian Fisheries Science*,**2008**, 20, 319–331
[22] A.O. Aniebo, E.S. Erondu., O.J. Owen. *Livestock Res Rural Dev*, **2008**, 20, 1–2.
[23] E.A. Fasakin, A.M. Balogun, O.O. Ajayi.*Aquaculture Research*, **2003**, 34, 733–738.
[24] Yi, L, C.M.Lakemond, L.M. Sagis, V. Eisner-Schadler, A. van Huis, M.A. van Boekel.*Food Chem*,**2013**,141(4), 3341-3348.
[25]D.M. Smith.Protein separation and characterization procedures. In: Food Analysis, 4th ed., Springer, US, **2010**, pp. 261-281.
[26] E. Pieterse, Q. Pretorius. *Animal Production Science*, **2013**, 54(3), 347-350.
[27] G. Wu.*Amino Acids*, **2013**, 45, 407-411.
[28]WHO, Protein and amino acid requirements in human nutrition, WHO Technical Report series 935, World Health Organization, Geneva, Switzerland, **2007.**
[29] P.W. Waldroup, J.H. Kersey, C.A. Fritts. *J PoultSci*, **2002**, 1, 136–144.
[30] K. Wilson, J. Walker J**.** Principles and techniques of practical biochemistry, 5th Edition , Cambridge University press, U K, **2000**; pp.770.
[31] H. B., Lauer, B. E. Baker.*Canadian Journal of Zoology*, **1977**, 55(1), 231-236.
[32] Z. Samicho, S. R. A.Mutalib ,N.Abdullah. *AgriculSc*, **2013**, 4(5B), 61-64.
[33]A. van Huis,J. Van Itterbeeck, H. Klunder, E. Mertens, A. Halloran, G. Muir, G. P. Vantomme. Edible insects - Future prospects for food and feed security. FAO Forestry Paper 171., FAO, Rome, **2013**; pp.154.