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Article

Determination of yellow mealworm (*Tenebrio molitor*) nutritional value as an animal and human food supplementation

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Abstract

For many decades insects have been used as food sources and supplementation due to their availability and easiness in rising that is much less burdensome for environment than animal husbandry and breeding. Mealworms are typically used as a pet food for fish and the birds. Additionally they are good for their high protein content. The aim of this study was to determine the nutritional value and chemical composition of mealworm (*Tenebrio molitor*) as afresh and sun dried larvae. Fresh and dried of mealworm contained 52.14 and 60.21% protein, respectively. This protein was also rich in amino acids such as Leucine, Lysine, Arginine and Serine. Fatty acid was detected with high value of Oleic acid, Linoleic acid and Palmetic acid in fresh and dried of mealworm. The determination of mineral content of mealworm are shown considerable amount of vitamins and minerals. The result of this study showed that fresh, dried and powdered larva is a high-grade product to be applied as a supplement to meals inclusion for animal and human nutrition.

Keywords yellow mealworm; chemical composition; amino acids; protein; fatty acid.

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1 Introduction

The Food and Agriculture Organization of the United Nation predicts that the human population will have grown to 9 billion by 2050, and these people will need a source of valuable food (FAO, 2012). The rapid growth of the human population in the second half of the 21st century may lead to shortages of food, especially animal proteins (Zhang et al., 2007; Zhang, 2008). The possible solutions to this problem were suggested to include the use of insects such as yellow mealworm as food for animal and human (WHO/FAO, 2007). It has

been reported that by insect's caloric value 50%were higher than soybeans; 87% were higher than corn; 63% were more than beef; 70% were higher than fish, lentils and beans; and 95% were higher than wheat, rye or teosintle (Defoliart, 1992). In many countries of South America and Africa, edible insects are habitually used as animal protein food for human consumption. However, people in the western world have, on average, a strong bias against insects as food, especially when the insects are offered in a recognizable form. Moreover (Taylor, 1975) reported the use of mealworms in a large scale as human food in many countries in Europe and Asia.

The Yellow mealworm beetles(*T. molitor*)are considered scavengers and are among the largest insects that infest stored products (Ghaly et al., 2009).Most prefer to feed on decaying grain or milled cereals in damp, poor conditions. These insects are usually found in places not frequently disturbed such as dark corners, under sacks, in bins and where feed is stored. Young larvae are white, darkening with age (Morgan, 1975; Lyons, 1991). Larvae of yellow mealworms are honey-yellow, while dark mealworms are dark-brown. Adults are shiny, dark-brown or black, whereas dark mealworm adults are dull, pitchy black. The larvae are best known as fish bait and as food for fish, amphibians, reptiles, turtles, birds, fowls and small mammals kept as house hold petsor in zoos. They are named as the best animal protein feeding stuff and reared in enormous quantities in small scale operations throughout the world for these uses (Ebeling, 1975).

Many researchers have investigated their content of minerals, vitamins, amino acids and fatty acids .They showed that fresh yellow mealworm larvae contain about 15% fat and 20% protein. Not only do mealworms utilize available food sources more efficiently than other livestock, they can also breakdown low-nutrient byproducts of common crops grown such as maize, wheat, millet and peanuts and quickly recycle them into high-quality food (Bodenheimer, 1951; Johnson, 2010; Bukkens, 1996).

There were several methods for oil and protein extraction from mealworm larvae, but the quality of the extracts is affected by the extraction procedure (Chen et al., 2010).To obtain protein as a food ingredient, many separation techniques are available on a laboratory scale that are generally based upon differences in protein solubility, size, charge, and biological affinities, e.g. salting out, isoelectric precipitation and solvent fractionation. Oil can be extracted by organic solvents or physical expelling, but safety and environmental andhealth issues have increased concern regarding industrial processes. Ethanol is a safe organic solvent and has been investigated for de-fatting soybeans and (*Quercussuber. L*) ethanol is also suitable as an extraction solvent for de-fatting food materials to be used for further protein extractions .In order to use protein from yellow mealworm larvae for human foods in a cost-effective way, protein extraction yield needs to be optimized and protein purity and functionality need to be characterized (Bodenheimer, 1951; Ghaly, 2009).Very little information in literature is available on extraction and isolation of extracted yellow mealworms protein, minerals, amino acids and fatty acids, therefore the aim of this study was to investigation the yellow mealworm larvae proximal components for consideration to use as a food ingredient and supplemental applications in animal nutrition.

2 Materials and Methods

Larvae of mealworm were obtained from insect culture carried on at and Entomology laboratory, Faculty of Agricultural and National Resources, Tehran Science and Research Branch, Islamic Azad University, Tehran, Iran. Insects were kept in plastic containers (35 × 25 × 20 cm) in 27±1˚C, on feed of oat flakes with addition of vegetables as a source of water. Portion of about 0.5 kg of three-month-old larvae being 26 - 32 mm in length was taken for analysis. One part was placed into refrigerator at 4˚C making larvae numbing, the other was submerged in boiling water bath for 20-30 second and dried in 60˚C. Both, fresh and dried larvae were milled for homogeneous mix. Materials were kept at 4˚C until used. The chemical analyses were performed at the

Faculty of Agricultural and National Resources, Tehran Science and Research Branch, Islamic Azad University, Tehran, Iran by using the techniques of the association of official analytical chemist (AOAC, 2000).

2.1 Moisture

To determine the moisture content of samples a method was used by drying the wet sample to a constant weight in an air circulating oven at 60-70^{o}C.

2.2 Protein, fatty acid and fiber analysis

Protein, fatty acid and fiber analysis was carried according to Randall, Soxtec and Diethylether Extraction-submersion method (AOAC, 2003). However different apparatuses were applied to precede analysis such as Foss kieltecanalyzer, Foss soxtec TM 2050 and ANKOM2000W for each crude protein, fat and fiber respectively. Ca 100-200mg sample was weighted 1-5 g test portions into tarred cellulose thimbles. While draining each portion, test portion was measured into thimble. The filter paper that used for washing test portion into thimble was taken and dried at 102± 2^{o}C for 2 hours. In order to use filtration, 1-2 gash, acid washed sand (EM SX0075-3, or equivalent CAS14808-60-7) or Celite (545) were additionally poured to bottom of filter or mixed in with test portion prior to water extraction. Prevention of solvent and test materials from absorbing extraction water-soluble components including carbohydrates, urea, lactic acid, and glycerol were considered. Defatted cotton (soak medical grade cotton in diethyl ether of hexanes for 24 hours, agitating several times during this period) was put before absorbing the melted fat in the pre-dry step. Also, it was possible to add cotton on top of test portion before 102 ± 2^{oC}, 2 h drying step. Insert three to four 5 mm glass boiling beads into each cup, and dry cups for minimum 30 min at 102 ± 2^{oC}.After transferring into desiccators and cooled down at room temperature, extraction cups were weighed to nearest 0.1 mg. Before attaching thimbles that contain dried test portions to extraction columns, extractor was preheated and condenser in cooling water had to turn on. While thimbles are in the boiling state, significant amount of solvent were applied to each extraction cup to cover test portion. The matches of cups with corresponding thimbles were checked after placed under extraction columns. Thimbles were reduced into solvent and boiled for20 min. For sample extraction completion, the critical reflux rate was verified. Thimbles were extracted up to40 min after raised out of solvent. In order to obtain solvent and attain apparent dryness, the possible amount of solvent was evaporated. Evaporating solvent was wrapped up when extraction cups (aluminum of glass, extraction temperature settings many differ; consult manufacturer's operating instructions) were removed from extractor and transferred into operating fume hood at low temperature. Moisture was removed by drying extraction cups in an oven set at 102º± 2^{oC} for 30 min; lastly, dry extraction cups were cooled in desiccators at room temperature and weighed to nearest 0.1 mg.

2.3 Amino acid, crude ash and minerals analysis

An amino acid, crude ash and minerals content of mealworm sample were performed by the methods of the Association of Official Analytical Chemists (AOAC, 2000).Hitachi L-8900 amino acid analyzer apparatus was used for amino acid analysis. Mineral samples were tested by using GBC Inductively coupled plasma integra XL ANKOM 2000W.Crude ash Vecstar Furnace division apparatus used performed for crude ash test. For the preliminary process, cruciform was burnt at electric stove 600 º C for 1-2 hours and then cooled down for 40 mins. After weighing, 2-3 g sample was taken and put into increased temperature an electric furnace or gas burner prior to next step. Sample was again placed in electric stove to burn for 2 hours and cooled down at desiccators for 40 min. After drying, the crude ash content was found by burned sample weight.

2.4 Microbiology analysis

Microbiology analysis showed that there were no detection of Escherichia coli (E.coli) and Salmonella spp. in larva. These result further supporting the possibility of introducing mealworm in human and animal food

consumption.

2.5 Statistical analysis

Results are the mean of determinations, and the standard deviation (SD) in reported. Results were analyzed by t-test.

3 Results

The ingredients and proximal contents of *Tenebrio molitor* larvae meal are shown in Table 1.

Table 1 Proximal content of *Tenebrio molitor* larvae (% Dry Matter Basis).

Components (%)	Fresh	Dried	t	df	P-value
Moisture	36.42±1.31	7.25±1.16	2	1, 7	0.0096
Crude Protein	52.14±0.90	60.21±1.08	2	1, 7	0.0082
Crude Fiber	24.36±0.54	22.35±0.59	2	1, 7	0.0020
Crude Ash	3.24±0.16	4.20±0.32	2	1, 7	0.0002
Crude Fat	28.38±0.48	19.12±0.26	2	1, 7	0.0023

The meal of *T. molitor* larvae that used in this trial had 36.24 and 7.25 moisture and contained 52.14 and 60.21 crude protein in each fresh and dried sample respectively (Table 2). Additionally it had 24.36, 2.35 crude fiber. The crude ash content was increased in sun dried samples but crude fat tended to decreased. The value results in agreement with those report by some researchers literatures (Finke, 2002; Makkar et al., 2014).The mealworm larvae contain fiber, which helps digestion, and which cannot be obtained from meat of farm animals (Ramos-Elorduy et al, 2008).

Table 2 Amino acid content of *T. molitor*larvae, (Grams per 100 g of protein).

Amino Acids	Fresh	Dried	P-value
Isoleucine (IIe)	1.72	1.83	0.0061
Leucine (Leu)	3.02	3.13	0.0003
Lysine (Lys)	2.41	2.50	0.0006
Methionine(Met)	0.50	0.52	0.0010
Phenylalanine(Phe)	1.44	1.55	0.0012
Threonine (Thr)	1.60	1.70	0.0030
Valine (Val)	2.36	2.57	0.0014
Histidine (His)	1.17	1.38	0.0019
Arginine (Arg)	2.02	2.23	0.0011
Threonine (Thr)	1.45	1.70	0.0023
Serine (Ser)	2.01	2.23	0.0009

Table 3 Mineral content of *T. molitor* larvae (mg of mineral per kg of sample).

Minerals	Fresh	Dried	P-value
Calcium(Ca)	514.12±10.26	500.12±10.21	0.0016
Phosphorus(P)	950.12±17.40	976.36±18.48	0.0023
Potassium(K)	932.63±12.51	953.20±16.65	0.0096
Iron(Fe)	65.36±0.98	68.20±0.99	0.0074
Magnesium(Mg)	1596.30±14.36	1630.14±18.36	0.0035
Zinc (Zn)	96.14±1.78	106.31±1.99	0.0021
Copper (Cu)	16.96±1.55	19.05±1.59	0.0083

The determination of mineral content of *T. molitor* larvae are shown in Table 3. Aguilar-Miranda et al (2002) and (Kirket al., 2000) confirmed that *T. molitor* larvae contain a considerable amount of vitamins and minerals.

Table 4 and 5 presents fatty acid contents in fresh and powder of *T. molitor* larvae. Fresh larvae contained significantly different amounts of myristic acid, palmitic acid, stearic acid, oleic acid, linoleicacid and linolenicacid than the dried and powdered one. Eicosanoid acid and Docosatetraenoic acid decrease in dried samples respectively.

Table 4 Fatty acids content of *T. molitor* larvae (grams per 100 g of sample).

Fatty Acids	Fresh	Dried	P-value
Myristic acid (C14:0)	2.99±0.65	3.26±0.72	0.0031
Palmitic acid (C16:0)	15.65±1.32	17.21±1.46	0.0042
Stearic acid (C18:0)	2.86±0.98	3.06±1.01	0.0050
Oleic acid (C18:ln9)	42.38±2.36	44.36±2.41	0.0001
Linoleic acid (C18:2n6)	32.01±1.67	31.63±1.36	0.0051
Linolenic acid (C18:3n3)	1.54±0.06	1.46±0.05	0.0036
Eicosanoid acid (C20:ln9)	0.43±0.02	0.39±0.01	0.0014
Arachidonic acid (C20:4n6)	0.44±0.06	0.50±0.08	0.0016
Docosatetraenoic acid (C22:4n6)	0.54±0.07	0.41±0.05	0.0020

Finke (2002) analyzed the phospholipids fatty acid composition of the adult *T. molitor* larvae and found that over 80 percent of these fatty acids consisted of palmitic, stearic, oleic and linoleic acids.

Table 5 Fatty acids content of *T. molitor* larvae (grams per 100 g of sample).

Fatty Acids	Fresh	Dried	P-value
Saturated fatty acid	22.30±1.21	23.34±1.28	0.001
Unsaturated fatty acid	76.19±2.12	78.41±2.64	0.002
Omega 3	45.54±1.16	47.25±1.28	0.006
Omega 6	31.12±0.88	33.06±0.96	0.004

The high content of fatty acids in insects has been well documented in the scientific literature. Additionally, the high content of fatty acids in diet affects its antioxidant activity, which is highly desirable in the human diet. The fatty acid profile of *T. molitor* larvae that was used in our study was in accordance with the results of Fine (2002) and Fontaneto et al. (2011) (Table 6).

Table 6 Bacterium content of *T. molitor* larvae (cfu per mg sample).

Fatty Acids	Fresh	Dried
Escherichia coli	Undetected	Undetected
Salmonellac spp.	Undetected	Undetected

4 Discussion

According to the nutritional value and content, the edible insects have been stated to have more of it when compared to other traditional diets. Insects are commonly consumed and they are considered to be highly food conversion efficiency compare the other animals (Belluco et al., 2013). Studies using dietary insects as feed ingredients were mostly focused on poultry nutrition, and showed that using them as a protein source in poultry diets had positive effects on their growth performance (Shen et al., 2006; Zhang, 2002).

Cromwell (1998) showed that animal protein sources have better availability compared to plant-derived protein sources because of the balanced amino acid composition in animal protein. Hwangbo and Hong (2009) demonstrated that broilers fed diet containing 30% housefly larvae meal had a higher apparent crude protein and amino acid digestibility than that of broilers fed basal diet. Belluco et al (2013) mentioned that it is important to note, first, that protein had the most significant reduction in digestibility in the Tenebrio molitor larvae diet when compared with the other tested chemical characteristics and, second, that the increase of indigestible protein in the Tenebrio molitor larvae diet can be ascribed to the proteins linked to chitin and therefore present in the insect exoskeleton. It was considered that the insect meal protein had a low content of Met, Cys, Lys, and Trp and that the cuticle proteins presented an amino acids composition different from that of the whole insect (Finke, 2007; Zhao et al.,2016).

Insects has a potential being an agent in recycling waste products and resources for highly nutritive diet for many other domesticated animals as well as for human consumption (Bukkens,1996). Lokeshwari and Shantibala (2010) demonstrated that the consuming insects for protein source would provide effective smaller amount and more ecological in contrast with vertebrate protein source. The result of (Capinera, 2004) study showed that the conversion of house cricket was twice as efficient as pigs and boiler chick, four times that of sheep, and six times higher than steer by estimating dressing percentage and losses in carcass.

In the Ng et al. (2001) study, catfish showed a good performance of growth and utilization efficiency when it fed on 80% of mealworm-based diet and they contained higher lipids content in their carcass after fed on mealworm-based diet. This data suggested that mealworm is highly nutritive diet and acceptable as an alternative protein source

Ramos Elorduy et al. (2002) used three levels of larvae (0, 5 and 10 percent dry weight) in a 19 percent protein content sorghum–soybean meal basal diet to evaluate feed intake, weight gain and feed efficiency in broiler chicks. After 15 days there were no significant differences between treatments. Mealworms are promising alternatives to conventional protein sources, particularly soybean meal.

Jin et al. (2016) showed that inclusion of dried mealworm up to 6% in weaning pig's diet is beneficial for

weaning pigs by improvement of growth performance. Dried mealworm supplementation increased feed intake and nutrient digestibility without any detrimental effect on immune response.

Kirk et al. (2000) showed that broiler chicks Ca content increased within 24 hours linearly and declined after a week, and this tendency was strongest with the highest levels of Ca supplementation. They also demonstrated that Ca amount in mealworms was 76% which was same as Ca in oyster shell.

Ramos Elorduy et al (2009) and Zhao et al. (2016) showed that composition of oils extracted from mealworms are rich in polyunsaturated fatty acids and frequently contain the essential linoleic and α-linolenic acids. The nutritional importance of these two essential fatty acids is well recognized, mainly for the healthy development of children (Michalsen et al., 2009).

5 Conclusion

In conclusion we could demonstrate that a larva of mealworm is one of the good sources as a human and animal's food. The high protein content of the mealworm larvae and the fact that this insect is easy to rear and maintain make the results of this study very interesting. The high moisture content of the mealworms (36%) could cause storage and handling problems and it seems that drying could reduce the problem. We demonstrated that the powdered larvae is a high-grade product to be applied as a supplement to food supplementation for animal and human nutrition, To make mealworms commonly used as human and animal food, it is necessary to develop the technology which will allow large scale productions at a reasonable cost. Because of amino acid composition, water and fat or oil absorption capacity, protein solubility, microstructure of the yellow mealworm protein extract dispersion and rheological properties are important attributes for the use of as a food ingredient, future studies are needed to identify and refine processing parameters that affects the functionality and quality of the protein and to determine how much of mealworm larva can use in animal nutrition as a supplement of their diets.

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