Mealworm – A Great Source Of Chitosan

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Abstract
The Tenebrio molitor (yellow mealworm beetle) larvae are known as mealworms. Tenebrio molitor belongs to the family Tenebrionidae. The rearing and production of mealworms can be done at a small space and only few resources are needed. Mealworms are being use as feed, food, source of chitin and chitosan, biofertilizers, bioactive compounds, for biodiesel production, and the use of mealworm frass. Mealworms can be a good source of chitin and chitosan. Many research have come forward with great extraction procedures for the extraction of chitin and chitosan from mealworms. Chitosan extracted from mealworm beetle showed antimicrobial activity. The demand for chitosan is increasing. Therefore, taking into account all the beneficial factors, mealworms can be used as alternative source for chitosan production.

Keywords: alternative source, chitosan, extraction, Tenebrio molitor, mealworms

Introduction

Chitin is abundantly available in nature. Chitosan is deacetylated derivative of chitin. Chitosan has a wide range of applications in major scientific and industrial areas like pharmaceuticals, biotechnology, medicine, agriculture, wastewater treatment and other [1-3]. Extraction of chitosan can be from different sources. The sources of chitosan include yeasts, bacteria, fungus, insects, algae and some other organisms and the shells of crustacean act as the most common source [4, 5]. Crab and shrimp shells are the major main natural sources of chitin. But they are available seasonally and cost effective. However, the demand for chitin and chitosan is increasing and it is important to check for other alternative of the sources. One great alternative is the used of insects for chitin and chitosan extraction and especially mealworms can be utilized for this purpose [6].

The *Tenebrio molitor* (yellow mealworm beetle) larvae are known as mealworms. The production of mealworm has small environmental impact, because few resources are required for rearing them [7]. Only a small space is required. For obtaining a kg of edible mealworm protein and the production of greenhouse gas is very small and lower than the rearing of chicken, cattle, and pigs [8]. Rearing of mealworm can be done in a wide range of substrates and by-products from food industry and in turn, change them into considerable bioconversion tool, food losses are being reduced and fitting into the concept of sustainable CE [9]. Many substrates that is several insect feeds were examined in mealworm rearing, focusing on certain plasticity of insect [10-14]. Simultaneously, many product types are being derived from the rearing of mealworm [6]. And also, many more products will be released in the next coming years probably [6]. Chitin and chitosan are important products of mealworms. The exuvium and whole body of mealworm larvae may be a source of chitin and chitosan for domestic animal feed [15]. Chitin from mealworm is found to have much softer texture from the crustacean chitin and have more better anti-inflammatory effects [16]. Mealworm chitosan is found to have similar structure with the commercially available chitosan and observed to show antimicrobial activity [17]. Mealworms can be used as a good source of chitosan production and in this review paper, mealworms description, uses of mealworms and the extraction of chitosan from mealworms are being focused.

Distribution of Mealworm

The larvae of yellow mealworm beetle (*Tenebrio molitor* Linnaeus, 1758) are known as mealworms. *Tenebrio molitor* is a species of darkling beetle belonging to the family Tenebrionidae. Mealworms probably originated in the Mediterranean [18]. Nowadays, its distribution worldwide because of colonisation and trade [18]. Historically, mealworms were known as a pest which affected the stored grains (molitor means "miller" in Latin) [6]. During the last decades, mealworms have been studied for different purposes. It may be for feed-food purposes and for waste management [6]. Mealworm larvae are being reported for rich source of nutrition. They are being reported to possess good amount of crude protein, crude fat, profile, essential and non-essential amino acids, fatty acids, vitamins and minerals. Larvae have good composition of minerals and vitamins like phosphorus, zinc, potassium iron, magnesium and copper [18, 19] and vitamins H, E, B3, B12, B5, and B2 [20]. The production of mealworm has small environmental impact, because few resources are required for rearing them [7]. For obtaining a kg of edible mealworm protein and the production of greenhouse gas is very small and lower than the rearing of chicken, cattle, and pigs [8]. Rearing of mealworm can be done in a wide range of substrates and by-products from food industry [9].

Description of Mealworm

The mealworm is a holometabolic insect. Mealworms has complete metamorphosis that is the 4 stages of life, egg, larva, pupa and adult. The mealworm, *Tenebrio molitor* L. is a big beetle of 15 mm long. It feeds on plant products causing damage to the total mass and nutritive value, after feeding on the stored foods, they contaminate with exuviates, excrements and dead insects. Eggs of *Tenebrio molitor* are ovoid, elongated and covered with some sticky substance, this sticky substance makes the eggs attaches to the substrate [22]. Small whitish larvae of about 3 mm long hatch from the eggs [22]. The whitish larvae after a few days, turns yellowish producing a hard and chitinous exoskeleton. When it becomes an adult larva, adult larva is of a length of 25 - 35 mm and its weight is about 0.2 g [23]. Some parts of the world use the adult larvae as human food [23, 24]. The pupa is a free-living creature which is of 12 - 18 mm in length and the colour is of creamy white. After 4 - 17 days after copulation, mealworms start laying eggs. A female can lay up to 500 eggs [22]. The optimum incubation temperature is 25°C - 27°C and the embryonic development can last 4 to 6 days [22]. If the temperature is increased slightly, hatching can be accelerated. *Tenebrio molitor* has a very long larval development which terminates after about half a year at the optimum temperature and low moisture condition. Larvae feed deeply in the products by avoiding the sunlight. Just before the completion of this stage, larvae appear onto the surface of food products and then, become pupae. The pupal stage may be 5 to 6 days at an optimum temperature.

Uses of Mealworm

Mealworm has several importance. Mealworms are being use as feed, food, source of chitin and chitosan, bioactive compounds, for biodiesel production and the use of mealworm frass. Mealworms are rich in protein and energy; thus, their larvae are being used as feed or meal and as an ingredient in other feed [6]. Feeds based on mealworms are used for feeding fishes, poultry, and for some animals. Insects have high nutritional value and sustainable production. Therefore, insects can be a feasible solution to encounter the increasing food demand for human consumption [25]. Many research have studied the utilization of mealworms as one of the ingredients in foods like bakery products and protein bars [6]. It has been reported that mealworm powder is used as a fortification component of bread [26]. The addition of mealworm powder of 5 to 10% helped in improving the softness and volume of bread, and also, protein and amino acids content of the bread [26]. Insects can accumulate saturated fatty acids that is C18 & C16 with physical and chemical properties [27]. Insects' fat was used for biodiesel production [28-31]. Mealworm has been studied and investigated to substitute oilseeds [31,32]. An innovative and environmentally friendly technology which consists of the application of *Tenebrio molitor* and *Hermetia illucens* for improving corn stover utilisation to produce biodiesel, defatted larval meal and biofertilizer has been employed [33]. The exuvium and whole body of mealworm larvae may be a source of chitin and chitosan for domestic animal feed [15]. Chitin from mealworm is found to have much softer texture from the crustacean chitin and have more better anti-inflammatory effects [16]. Mealworm chitosan is found to have similar structure with the commercially available chitosan and observed to show antimicrobial activity [17]. Frass is the generic term which refers to insect larvae' excrements or the mix of them with the rearing substrate. The frass from the production of mealworm larvae can be utilised in different ways, it can be used as an organic fertiliser [34,35]. And frass can be utilized as a substitution whether partially or completely of mineral fertilizer NPK (nitrogen, phosphorous and potassium) where the mineral fertilizers availability is very limited [36].

Chitosan and its Applications

Chitin is aminopolysaccharide polymer abundantly available in nature. Chitin is the building substance which provides strength to the exoskeletons of insects, crustaceans and also to the cell wall of fungi. Chitin is found in crustaceans and it is present as part of a complex network of proteins on which accumulation of calcium carbonate occurs and forms the hard shell [37]. Chitin could be converted to its derivative, chitosan by enzymatic or chemical deacetylation. Chitosan is a modified carbohydrate polymer obtained by hydrolysing the aminoacetyl groups of chitins [38]. Chitosan is one of the most abundant materials in the world and is renewable [37]. Chitosan is derived from the deacetylation of chitin and it the second most abundant natural biopolymer and can be extracted from crustacean like crabs and shrimps and also from insects and fungus [39]. Chitosan is nontoxic copolymer which is consisted of β-(1,4)-2-acetamido-2-deoxy-d-glucopyranosyl and β-(1,4)-2-amino-2-deoxy-d-glucopyranosyl units [40]. Chitosan has many beneficial properties like antimicrobial, biocompatible with less toxicity [40]. Because of the non-toxicity, antimicrobial, environmental compatibility, biocompatibility and safety nature, chitosan has several applicability such as in biology, cosmetics, food, photographic chemistry, dyeing, paper making, printing, wastewater treatment and heavy metal recovery [41]. Antimicrobial activity study for the chitosan extracted from mealworm beetle was observed to show inhibition against *Listeria monocytogenes*, *Escherischia coli, Staphylococcus aureus* and *Bacillus cereus* [17]. Chitosan showed zones of inhibition against *Pseudomonas aeruginosa* [4]. A chitosan oligosaccharide which has a degree of polymerization of 6 to 8 have been found to have antitumor activity [42]. The interest on chitosan has been increasing for its applications in veterinary medicine, chemistry, textiles, dentistry, cosmetics, environmental science, biotechnology, and medicine [43]. Recently, the utilisation of chitosan in agriculture has become prominent and chitosan and oligo-chitosan activity on several plant insects and pathogens were studied [37].

Extraction of Chitosan from Mealworms

The mealworm beetle (*Tenebrio molitor*) does not require a vast production area. Demoralization is the first step for the extraction of chitin, it is removal of calcium carbonate which is usually performed by hot reaction using HCl, HNO_3 etc and followed by deproteinization [37]. Song et al., (2018) investigated chitin and chitosan production from the exuvium and whole body of mealworm (*Tenebrio molitor*) larvae. The average demineralization percentage and deproteinization percentage was found to be 32.56 and 73.16 percent from larval exuvium and from whole body was 41.68 and 91.53 percent on a dry weight basis, respectively. Then, for converting to chitosan, exuvium and whole body chitin particles of *Tenebrio molitor* larva were heated at various temperatures taking different concentrations of NaOH. Chitin yields were reported to be 18.01% and 4.92% of dry weight from the exuvium and whole body, respectively. They obtained the average chitosan yield from whole body to be 3.65 percent of the dry weight. Their results suggested that *Tenebrio molitor* larva exuvium and whole body larva may serve as a source of extraction of chitin and chitosan. Chitin yields of the adult, larvae, and superworm of mealworm beetle were obtained to be 8.40, 4.60, and 3.90%, respectively [17]. And these chitin yields were converted to chitosan by deacetylation and chitosan yields were obtained to be 83.33, 80.00, and 78.33 % in the superworm, larvae, and adult of mealworm beetle, respectively [17].

Nafary et al. [4] extracted chitin and chitosan from *Tenebrio molitor* by the application of two different methods. The dried *Tenebrio molitor* were grounded to powder and then passed through a 250 m sieve. For the first method, the dried powder sample was demineralized using hydrochloric acid for two hours at 65 to 75°C for chitin extraction and the solution was filtered. The filtrates were kept in NaOH at 80 to 90°C and filtered again and washed with deionized water for the removal of any protein residues. Then for decolorization, the filtered were placed in a solution of chloroform, methanol, and water (with 1:2:4 ratio) for an hour and mixture was filtered and washed with distilled water and thus extracted chitin was kept at 60°C [44]. For the second method, the dried powder sample was refluxed at 100°C for 10 minutes in sodium hypochlorite solution and after rinsing with distilled water, the repetition of this step was done. For demineralization, the samples were refluxed for 15 minutes in HCl at 75°C. Then the reflux of the samples was performed NaOH solution at 100°C for 20 minutes for deproteinization and after the final extraction of the sample, the extracts were dried at 60°C [45]. Then follows the extraction process for chitosan, for the conversion of chitosan from chitin, 1 gram of each of the extracted chitin from *Tenebrio molitor* was taken and deacetylation process was done by the treatment of the sample with 50% NaOH (weight/volume 1:20) at 100°C for 3 hours. Then, the samples were washed using deionized water to get pH 7 and chitosan samples thus obtained were kept for drying at for 24 hours 40°C. The yields of the chitin were observed to be 13.3 and 17.7% from the dry mealworm and yield of chitosan from the extracted chitin were found to be 78.26% and 76.43%, respectively for the first and second method [4]. Their result showed higher chitin yield was observed from that of a report by Hardani et al. [46] where the chitin yield from crab and shrimp were 52.63% and 45%.

Conclusion

Chitosan is deacetylated derivative of chitin and have a wide range of applications in major scientific and industrial areas like pharmaceuticals, biotechnology, medicine, agriculture, wastewater treatment and other. Crab and shrimp shells are the major main natural sources of chitin however, they are available seasonally and cost effective. So as to meet the increasing demand for chitin and chitosan, the other alternative of the sources is the insects. Extraction of chitin and chitosan from mealworms give a better yield. Chitosan extracted from mealworms may replace commercial chitosan and mealworms can be used an alternative of crap and shrimp for the extraction of chitin and chitosan.

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