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Effect of pre-treatments on proximate composition, protein extraction yield and extraction rate of Superworm protein

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ABSTRACT: Superworm (*Zophobas morio*) is the larval stage of darkling beetle which is rich in protein and fat. Three different drying methods, Viz., freeze drying, hot air oven drying, and microwave-assisted hot air oven drying, were used as a pre-treatment for insect drying. Freeze drying was the most suitable method as both extraction rate and the yield of superworm protein were higher. In freeze drying, the least damage is caused to protein content compared to the other two drying methods. The proximate analysis was performed for freeze-dried insect powder and extracted superworm protein powder, which showed the total protein content of superworm insect is 53.32±0.54%, and extracted protein from superworm insect has a protein content of 85.29±0.13%. Along with protein fat content of superworm (40%) was significantly higher. Colour values L^* a^* b^* were positive for freeze-dried superworm powder and extracted protein. Extracted superworm protein (L^*79.02±0.00) has a lighter colour than freeze-dried superworm insect powder (L^*43.26±0.01). Water activity for freeze-dried superworm insects and freeze-dried superworm insect protein 0.41 and 0.21. Lower values of water activity have marked the shelf stable nature of freeze-dried insect powder as well as superworm protein powder. The major challenge for this study was the procurement of superworm larvae and maintaining them in a proper atmosphere. Superworm can be an excellent alternative protein source that can be used to combat protein energy malnutrition worldwide.

Keywords: Superworm, Protein, Extraction yield, Extraction rate, Water activity.

INTRODUCTION

Entomophagy is the practice of consuming insects (Niveditha *et al.,*2021). The term "entomos" means insect and “phagein” means to eat (Parvez, 2017). By the year 2050, there will be around 9 billion people on the planet. We must find new protein sources to fulfil this population's increasing protein energy need s (Iseppi *et al.,* 2021). According to FAO, there are around 821 million malnourished people around the globe. In such instance, food insecurity may emerge, andinsects might serve as an alternate food source for the whole population.(Yen, 2009) showed the importance of sustainable harvesting of edible insects to use as food. To prevent the unfavourable environmental impacts of livestock production, insects can be used as an alternative source of proteins for humans. Depending on the kind and developmental stage of the insect, the dry matter protein content ranges from 20 to 76%. Large variations in fat content (dry matter of 2 to 50%) occurs depending on developmental stage in superworm larvae will have more fat content than adult.

Superworm is often raised for use as food for fish, birds, and reptiles. Superworm is a member of the Tenebrionidae giant beetle family which also includes red flour beetle, confused flour beetle, and other stored commodity insects, including *Alphitobius diaperinus,* and *Tenebrio molitor* (Coleoptera: Tenebrionidae). Protein and fat are two nutrients abundant in superworm larvae. Superworm has a protein content of 47-48% (Kulma *et al*., 2020). Although listed among storage insects, *Z. morio* is associated with only one stored commodity, *i.e.,* wheat flour (Rumbos & Athanassiou 2021). The larvae are yellow with dark brown ends on the front and back. Their exoskeleton is cylindrical, heavily sclerotized, and conically constricted between the seventh and ninth abdominal segment bases. At 25 °C, they may hatch after 8 days and grow to a maximum length of 55 mm. The number and length of larval instars are density-dependent, meaning that they vary depending on whether larvae are kept in groups or isolated environments. When maintained apart, larvae pupate between 11 and 18 instars; however, most pupations occur between 16 and 17 moults. This species fails to pupate under crowded conditions, despite continuing larval moults till death is one of its most significant traits. The rate of pupation slows down as larval density rises. This effect is ascribed to the mechanical stimulation caused by interactions between larvae rather than being pheromone-mediated or produced by auditory or visual stimuli (Rumbos &

Athanassiou 2021). According to Australian researchers, the solution to recycling plastic may lie in superworms that eat polystyrene (Sun *et al.,* 2022). Several recent studies (Alves *et al.*, 2021; Abd Rahman Jabir, 2012; Rumbos & Athanassiou 2021) have disclosed better results of superworm meal on the fishes as compared to traditional fish meal. Recently, the superworms whole mitochondrial genome was sequenced, indicating that researchers are becoming more interested in this species (Bai *et al.,* 2019).

The present study has been undertaken to estimate the effect of different pre-treatments on proximate composition, protein extraction yield and protein extraction rate of superworm.

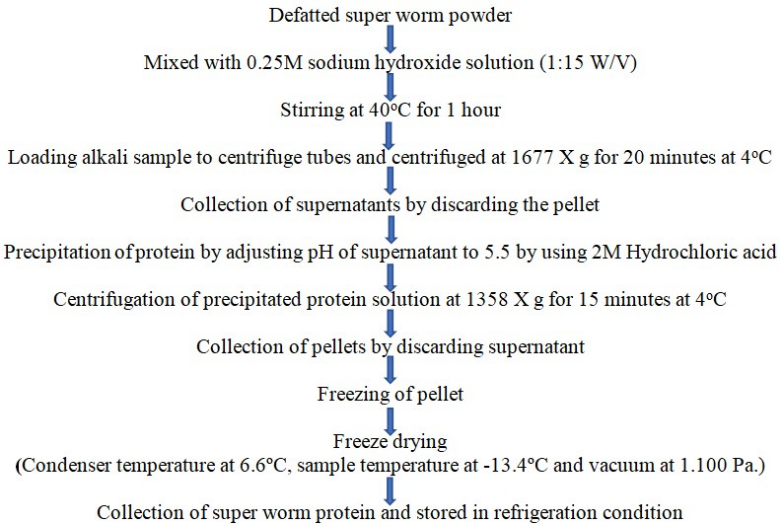
MATERIALS AND METHODS

The live superworm larvae were purchased from mealfarm® Bengaluru, Karnataka, India. Superworm larvae were held at – 4°C for 1 hour to kill them, followed by drying with three different methods.

1. Freeze drying (condenser temperature 6.6°C, sample temperature -13.4°C, and vacuum at 1.100Pa).
2. Drying at 70°C in a hot air oven for 48 hours.
3. Microwave-assisted hot air drying (60°C, 60 min, 3 kilowatts).

Dried insects were pulverized to powder it. The powdered insects were stored at 4°C in the refrigerator. Powdered insects were defatted using n-Hexane as a solvent before protein extraction.

A. Method of Protein Extraction



B. Extraction rate and Extraction yield

The extraction yield and rate of protein were calculated using the following formulae (Zhao *et al*., 2016).

$$\text{Extraction yield (EY)} = \frac{Weight\ of\ extract}{Weight\ of\ sample} \times 100$$

$$\text{Extraction Rate of protein} = \frac{\text{Protein content in extract}}{\text{Protein content in sample}} \times EY$$

C. Proximate Analysis

Analysis of protein content was done for all three pre-treatments as it is necessary to calculate protein extraction yield and protein extraction rate. Remaining proximate analyses such as ash, crude fibre, fat, carbohydrates, colour and water activity were only done for pre-treatment with higher protein content, protein extraction yield and protein extraction rate.

The proximate composition of superworm larvae for protein, ash, crude fibre, fat, colour, and water activity was tested in triplicates based on the Association of Official Analytical Chemists international method (AOAC, 2019).

Moisture content (934.01) was measured using the hot air oven technique by drying a 5g sample at 105°C for 4 hours. Protein content was evaluated using the Kjeldhal method (984.13) and a utilized nitrogen conversion factor of (6.25 × N) (Gosukonda *et al.,* 2020). Ash (942.05) was measured by burning the dry sample (5g) in a muffle furnace at 650°C for 2 hours. For fat (920.39) estimated 2g sample was obtained in a thimble put in the Soxhlet apparatus, and N-Hexane (60-65°C) was used as a solvent. Crude fibre (962.09) was measured by processing the defatted dry sample (2 g) washed in 1.25% HCl and 1.25% NaOH followed by filtration and final residue collection and weighing. Carbohydrate content was determined by Moisture, ash, crude protein and crude fat were subtracted from 100 to get the total carbohydrate (Wadje & Meenatchi 2022).

D. Analysis of colour values

The colour parameters (L^*, a^*, b^*) of superworm powder were calculated using a Hunter lab colourimeter (ColorFlex EZ 45/0-LAV, Hunter Associates Laboratory Inc., Virginia, USA). It operates on the principle of gathering light and measuring energy from a sample that has been reflected over the visible spectrum. Each time, the tools were standardized with colours like white and black. Samples were scanned to determine the L*, a* and b* where L* indicates

lightness and darkness, a* indicates red (+a) and green (-a), b* indicates yellow (+b) and blue (-b) (Shashikumar *et al*., 2021).

E. Analysis of water activity

The ratio of the vapour pressure of the water above the sample to the vapour pressure of pure water at the same temperature is known as water activity (aw) at a specific temperature. It is a significant parameter that provides information regarding products stability, quality and microbial safety. The aw was determined by using dew point water activity meter (AquaLab 4TE, Inc. Pullman, WA, USA) with ± 0.001 sensitivity. The setup determines the dew point temperature of the sample precisely using an infra-red beam which is being focused on tiny mirror. Water activity of the sample was measured by placing inside the water activity meter in a disposable cup and the chamber was closed and set aside to equilibrate. The values displayed digitally were recorded.

F. Statistical Analysis

All the analysis for proximate composition, colour values, water activity and effect of drying method on protein content of superworm were performed in triplicates as Mean±Standard deviation. Effect of different drying methods on protein content of superworm insect were analysed using ANOVA in the (Minitab 18.1 statistical analysis tool software USA). Where results show the significant interaction between the samples and significant main effects at $p<0.05$. A comparison was done with post hoc approach Tukey with a confidence level of 95% for the analysis.

RESULTS AND DISCUSSION

A. Effect of pre-treatments on protein content

The protein content of insects dried using the freeze-drying method showed the highest amount of protein content (53.32±0.54%) as compared to hot air oven (50.95±0.72%) and microwave-assisted hot-air oven drying (51.49±0.08%). There was no significant difference between the protein content of insects dried using hot-air oven drying and microwave-assisted hot air oven drying. Whereas significant difference was there in the protein content of insects dried using freeze-drying and hot air oven, microwave assisted hot-air oven drying. The protein content of extracted superworm protein for the freeze-drying, hot air oven drying, and microwave-assisted hot air oven drying were 85.29±0.13%, 80.62±0.44%, and 84.08±0.09%, respectively. For all three pre-treatments there was a significant difference in the protein content of extracted superworm protein. The freeze-drying method is recommended for the high protein and high fat-containing products (Hu *et al*., 2013). Protein denaturation occurs while drying the high protein-containing food products using the hot air oven drying method. Slightly better results for protein content are seen in microwave-assisted hot air oven drying. The microwave drying technique improves colour, vit B2 and mineral content more effectively. Compared with hot oven drying, microwave drying requires a short processing time. Microwave drying will also improve the economic weight of the insects (Bawa *et al.*, 2020). Microwave consumes less energy, time and retains both nutritional and sensory attributes, provides pleasant aroma and bioactive components. Combined with the hot air oven drying technique, it gives better efficacy. During preliminary studies, drying at 60°C for 60 minutes, 3 kilowatts gave the best results with desirable colour and aroma. It was the maximum voltage that the microwave-assisted hot air dryer could attain.

Freeze drying method yielded highest protein content. Also, when dried with freeze-drying protein suffers minimum damage. The extracted protein should be undamaged to the extent possible when it is further characterised. A literature survey found that when a substance higher in protein dried using hot-air oven or microwave-assisted hot air oven drying, the protein in it undergoes structural damage.

Table 1: Effect of different drying methods on the protein content of superworm insect.

Sr. No.	Drying Method	Protein Content of superworm (%)	Protein content of extracted superworm protein (%)
1.	Freeze drying	53.32±0.54[a]	85.29±0.13[a]
2.	Hot-air oven drying	50.95±0.72[b]	80.62±0.44[b]
3.	Microwave-assisted hot air oven drying	51.49±0.10[b]	84.08±0.09[c]

Note: Values sharing different letters are significantly different ($p \leq 0.05$).

B. Protein Extraction Yield and Extraction Rate

The protein extraction yield and protein extraction rate depend on protein content of extracted protein and material from which protein is to be extracted. To increase the protein content all the samples were defatted using n-Hexane as a solvent (Choi *et al*., 2017). To assess the extraction process's efficiency and the product's economic worth, yield value calculation is a crucial factor (Haryati *et al*., 2019). The extraction yield (%) and extraction rate (%) of all three samples were calculated and presented in Fig. 1. The protein extraction yield was high in freeze-dried samples (43.73%). The reason may be due to the use of low-temperature proteins remain undamaged and because of alkali at low concentration proteins remained unhydrolyzed. Protein yield results of yellow mealworm (50.6%) were higher than superworm (Zhao *et al*., 2016). Next to freeze drying highest protein extraction yield was found in the microwave-assisted hot air oven dried sample (42%) and the least protein extraction

yield was found in the hot air oven dried sample (40%). Slight denaturation of the samples while pre-treating the sample lowers the nutritional value. In the hot air oven dried sample denaturation of thermally labile, oxidation sensitive nutrients, flavours and biologically active molecules occurs. While they are retained in microwave treated sample which resulted in high sample yield as compared to the hot air oven dried sample.

The rate of protein extraction was found to be highest in the freeze-dried sample (69.94%). The extraction rate of protein in freeze-dried and microwave-assisted hot air oven dried sample (68.55%) were more or less similar. But the protein extraction rate in hot air oven dried sample (63.29%) is less because the proteins suffer damage. The extraction yield and rate for freeze drying pre-treatment were higher because the protein content of both freeze-dried insects and extracted superworm protein was higher.

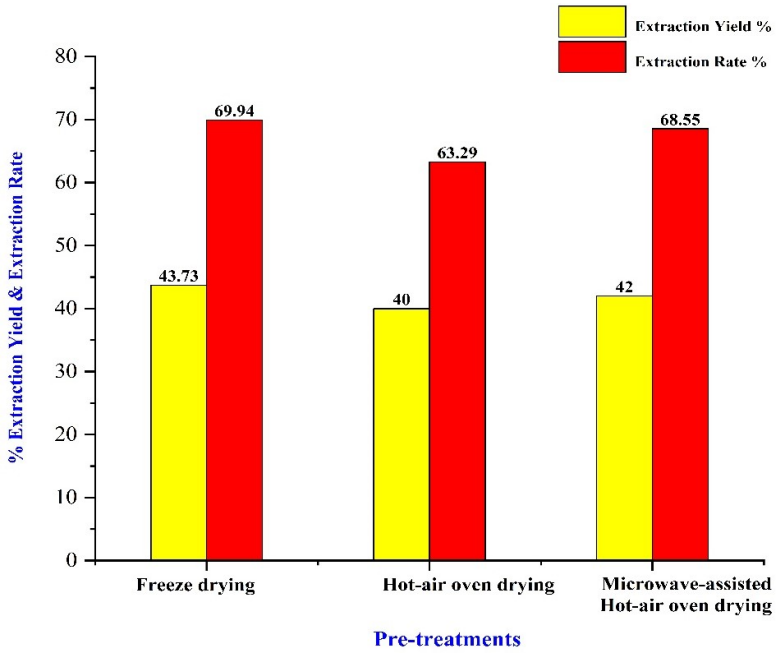


Fig 1. Effect of pre-treatments on extraction yield and extraction rate.

C. Proximate analysis

Analysis of proximate composition is regarded as a principle criterion in determining the nutritional value and quality of the sample (Ogunyinka *et al.*, 2017). The proximate composition of superworm insect and extracted superworm protein were analyzed in triplicates and the results are represented in Table 2.

Crude protein content in the superworm insect is 53.32±0.54%, and the crude protein content of protein extracted from superworm is 85.29±0.13%. As protein content in superworm and protein extracted from superworm insect is higher, both resulted in a high protein extraction yield and protein extraction rate. The protein content of superworm is more than *T. molitor*, *Bombyx mori*, *Apis mellife*ra, *Hermetia illucens*. Some insects which have higher protein content than superworm are *A. diaperinus*, *Acheta domesticus*, *Blaptica dubia* etc., (Ghosh *et al*., 2016; H *et al*., 2021; Igual *et al.*, 2020; Lam *et al*., 2021; Roncolini *et al*., 2020; Soares Araújo *et al*., 2019; Wu *et al*., 2021). Superworm larvae are high in protein content as compared to adult darkling beetle. Larvae are more convenient for extracting protein than adults because adults will have more chitin content (Kaya *et al.*, 2016) and chitin interferes with the protein extraction. Superworm insect has a very high fat content (40%).

Table 2: Proximate composition of superworm insect and its protein.

Sr. No.	Parameters	Superworm Insect (%)	Superworm Protein Powder (%)
1.	Moisture content (wb)	3.32±0.001	0.16±0.22
2.	Protein (db)	53.32±0.54	85.29±0.13
3.	Fat (db)	40±0.05	2.01±0.46
4.	Ash (db)	0.23±0.007	0.29±0.4
5.	Crude fibre (db)	2.30±0.15	1.42±0.02
6.	Carbohydrates (db)	1.15±0.03	10.77±0.10

Protein extracted from superworm has less fat content (2.01±0.46) as it is extracted from defatted superworm powder. Silkworm pupae, one of the most popular edible insect has oil content of only 8%, which is much less than the superworm insect (Longvah *et al*., 2011). Further studies can be done to undiscover the potential of superworm oil. Superworm oil has low antioxidant activity and almost negligible antibacterial activity (Pumnuan *et al*., 2019). Iodine value, peroxide value and free fatty acid value of superworm oil will give the idea about its suitability to be used in the food and feed systems. Crude fibre (2.30±0.15) and carbohydrate content (1.15±0.03) of superworm protein was negligible.

D. Analysis of Colour Value

Colour plays a vital role in the evaluation and preference of food. In sensory analysis, most easily evaluated characteristic is colour. Colour values were measured to differentiate the impact of different pre-treatments and to estimate the colour of superworm powder and superworm protein. Colour values can be used to estimate the colour pigment content of extracted edible insect protein. L^* is for lightness and other two-colour channels $a^*$and $b^*$are known as chromaticity layers. Positive values of L^*, $a^*$and b^* depict lightness, yellow and red hues. Whereas negative values of L^*, $a^*$and b^* show Dark, blue and green shades. Since L^* of superworm insect powder is 43.26±0.01 which shows the darker colour of superworm powder but L^* value of superworm protein is 79.02±0.00 which shows extracted protein has a very light shade. The values for superworm insect powder and superworm protein showa slightly darker shade. As a^* values are slightly on the positive side it depicts the slight reddish tint for both the superworm powder (5.31±0.008) and superworm protein (2.24±0.008). Value of b^* is positive for both samples, which shows a slightly yellowish hue of superworm powder (13.97±0.00) and superworm protein (17.13±0.008). ΔE (Total colour differences), represent the magnitude of colour difference between the samples. ΔE value for superworm insect powder and superworm protein powder is 0.03. As ΔE values are negligible, total colour difference is also negligible for both samples. (Bellary *et al*., 2016) states that samples having ΔE > 30 the colour difference is perceivable to the naked eye. ΔE value between the superworm insect powder and superworm protein powder is 17.14 which marks the huge colour difference between the two. The colour difference might be due to various pre-treatments given such as defatting, grinding, freeze-drying. Generally, colour of insect based product will be decided by pigments such as melanin (WittKopp & Beldade 2009) and chitin (Battampara *et al.*, 2020). Colour values are dependent on pH conditions, protein aggregation and colour pigment(Atkinson *et al*., 1973). Colour values of both freeze-dried superworm powder and superworm protein were calculated and represented in Table 3.

Table 3: Colour values of superworm powder and superworm protein.

Sr. No.	Parameters	Superworm Insect Powder	Superworm protein
1.	L^*	43.26±0.01	79.02±0.00
2.	a^*	5.31±0.008	2.24±0.008
3.	b*	13.97±0.00	17.13±0.008
4.	ΔE	0.03	0.03

E. Analysis of water activity (aw)

aw is the measure of free water present in the product. aw deals with the water not bound to any cell component and is available for various microbial growth and chemical reactions. Water activity plays a fundamental role in assessing the stability of raw or processed food products. In freeze-dried products, aw will be reduced to a much lower extent (less than 0.5), which limits food spoilage and improves food safety by reducing microbial growth rate, chemical and enzymatic reactions. The aw of superworm dried using freeze-drying and powdered subsequently showed aw of 0.47±0.00. The aw of precipitated protein pellets and freeze-dried subsequently showed aw of 0.21±0.00. aw less than 0.7 shows that it may have longer shelf life at ambient temperatures and is termed safe moisture content (Jovanovich *et al.*, 2003). The aw of superworm protein is higher than whey protein isolate sample is 0.13 (Erdem & Kaya 2021), chickpea protein isolate is 0.179 (Tontul *et al.*, 2018) but lower than pea protein isolate, which is 0.6 (Mehle *et al*., 2020). The aw of superworm protein is similar to soya protein 0.22 (Mehle *et al*., 2020). aw of both the samples were less than 0.7, So we can conclude that both have a longer shelf life at ambient temperature.

CONCLUSION

Among the three different drying methods used, freeze-drying was most suitable method for insect drying because protein suffers the minimum damage, also, protein extraction rate and extraction yield were higher for the freeze-dried sample. Protein extraction yield and protein extraction rate were higher because of the higher protein content of insects as well as extracted protein from superworm insects dried using freeze drying. Superworm larvae are higher in lipid content. Extracted protein has a lighter shade as compared to freeze-dried insect powder. Since in colour analysis of superworm powder and superworm protein powder all the values of L^*, $a^*$and b^* were positive, all the colour shades lie in the light, yellow and red shades respectively.aw is a crucial factor in monitoring hygroscopic goods or

materials. Since the aw of the freeze dried superworm and freeze dried superworm protein powder is less. As aw for both the samples is less than 0.7, they can be stored at ambient room temperature. Nutrients such as protein and fat are rich in superworm. For further characterization studies freeze-drying pretreatment can be used as protein suffers least damage as compared to other drying method. Further characterization study can be done a superworm oil as it is rich in oil content.

FUTURE SCOPE

Characterization study can be done on extracted superworm protein and superworm oil recovered during defatting process to check their suitability to be used in food and feed systems.

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