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Utilization of black soldier fly (*Hermetia illucens*) prepupae flour in development of bio-packaging film

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Abstract

The black soldier fly with high nutritional value reared from food waste were used to develop bio-packaging film for food commodities. The plastic is a major concern in today's population. Thus to overcome this issue bio-packaging film was developed using insect flour. The black soldier fly prepupae have prominent amount of protein (42-44%) and chitin (9-11%) compound which can be used to develop bio-packaging films. The development of bio-packaging film from novel sources such as insects provides an opportunity to explore the under-utilized source. The insect flour bio-packaging was evaluated its suitability in food application by testing its tensile strength, antioxidant activity, opacity, color, water activity, and GSM. The film developed with 4% (w/v) black soldier fly prepupae flour in 1% (w/v) chitosan solution. The combination of flour and chitosan in 30:70 showed good mechanical properties. Decreasing the chitosan concentration significantly reduces the antioxidant property of the film The addition of glycerol exhibited glossy transparent appearance in control film whereas in other film composition were appeared to be in slight brownish due to the pigment present in flour component. The study concluded that exploring the nutritional rich black soldier fly prepupae as a sustainable resource of bio-packaging film in food sector with less frugal for futuristic mass commercialization.

Keywords: *Hermetia illucens,* defatted flour, novel film, sustainability, chitosan, bioplastic

1. Introduction

The black soldier fly (*Hermetia illucens*) emerged as a sustainable and alternative protein and chitin source to meet the futuristic population demand (Liu *et al.*, 2020) [4]. The black soldier fly (BSF) prepupae flour contains promising nutritional properties with approximately 42-44% crude protein, 31-35% crude fat, and chitin content 9-11% (Müller *et al.*, 2017; Purschke *et al.*, 2017) [16, 19, 21]. Thus currently black soldier fly prepupae (BSFP) emerged as an alternative protein and chitin source. The rearing of black soldier fly can be done from organic wastes (food waste-utilization) at low cost, less water and energy. This concept troubleshoots both waste management and alternative source of protein and chitin from insects for next-generation (Wang and Shelomi *et al.*, 2017; Nana *et al*., 2019) [30]. The biopolymer can be made from underutilized renewable insect sources which give better optimistic characteristics of the polymer since it have protein and chitin compounds. Recently many researchers approach studies on sustainable, composite and edible packaging as the scope of this area to utilize the abundant availability of fossil fuels and to prevent environmental pollution caused by synthetic polymers (Silva *et al.*, 2020) [24]. Thus in subsequent years, there will be increased growth in the production of bio-packaging. The major advantage of a biopolymer is leaving fewer carbon footprints in the environment. It can be made from renewable resources as a waste utilization (Álvarez-Chávez *et al*., 2012) [1]. The biodegradable and sustainable polymer in food packaging produces a greener future by reducing the use of non-biodegradable petroleum-based polymers and disposal of waste (Tharanathan *et al*., 2003) [25].

Recently many types of packaging evolved in market such as intelligent packaging, smart packaging, active packaging, ergonomics packaging, MAP & CAP. The major function is to protect the food from physical, chemical damage and food-borne diseases. In general food package act as a shield, restrain and deliver information about the product to the consumer to ensure the quality and safety of the food product (Vimala Bharathi *et al*., 2018) [3]. Currently, few polymers are primarily used in food packaging due to its versatility function such as LDPE, HDPE, PET and other materials. It is estimated that in 2030 the amount of plastic production will be doubled to 600 million tons (Kumar *et al*., 2018; Thompson *et al*., 2009) [13, 28]. The major negative impact of the polymer packaging material is environmental pollution and migration to food substances which lead to hazardous health issues. To overcome this issue, novel biodegradable packaging evolved which is sustainable and eco-friendly green packaging (Silva *et al.*, 2020) [24]. The most revolutionary concepts in the field of packaging technology are environment friendly and biopolymers. Biopolymers are derived from renewable resources such as plant-based and animal-based origin. The biodegradable edible packaging is made from bio-based polymers which are directly obtained from the renewable resources such as naturally derived polysaccharide, protein and lipid origin sources (Tharanathan *et al*., 2003; Silva *et al.*, 2020) [25, 24]. In this context special attention was provided by the FAO to potential novel alternative proteins from insect sources (Halloran *et al*., 2018; Cullere *et al*., 2019) [10]. Hence considering importance of environmental issue, alternative to plastic packaging this project is to develop an eco-friendly bio-packaging by utilizing underutilized insect sources such as black soldier fly prepupae flour added in chitosan matrix. The interesting advantage is raw material was sustainable and also low processing cost due to waste-utilization of organic matters for insect feeding purpose and further to develop a insect flour based bio-degradable packaging at low cost. Overall the concept of waste to wealth can be accomplished with less frugal.

2. Materials and Methodology

The black soldier fly (*H. illucens*) was reared in IIFPT-Thanjavur Entomology laboratory. All the chemicals used were analytical grade. The chitosan and glycerol was purchased from Sigma-Aldrich (India). The n-hexane was purchased from HiMedia (India).

2.1 Rearing of black soldier fly prepupae from food waste

The grown adults were employed in subsequent culturing. In an insect cage, the adults were allowed to lay eggs. The insect culture cage was set up with cardboard for the better oviposition of the BSF pupae as mentioned by Danieli *et al.*, 2019 [8]. Later, the eggs and feed substrate were carefully transferred into the culturing container. The larvae took 3-4 days to emerge from the eggs and were fed kitchen garbage such as wasted grains, vegetable & fruit waste as suggested by Devic *et al*., 2018 [9]. Then the larval (length about 15 mm) growth was carefully observed along with the feed substrate. Within a week, the larvae had progressed to the prepupal stage (length about 23 mm) (Barragan-Fonseca *et al*., 2018) [2].

2.2 Preparation of defatted Insect flour

The pre-pupae of BSF were removed out from the culturing container. The BSFP was pre-treated using a screening mesh to exclude the feed. The BSFP was thoroughly cleaned under running tap water. The sample is kept at -18 °C was used to terminate the live BSFP and stored at -18 °C in a deep freezer. The frozen BSFP were then lyophilized at -80 °C for 24 hours. The dried prepupae flour was pulverized and sieved through a mesh sieve (0.34mm) using sieve shaker. Then insect flour was subjected to defatting process using n-hexane as a solvent in soxhlet apparatus for 6 hours. After being defatted with n-hexane, the defatted insect flour is stored in vacuum-sealed pouches at -18°C (Bubler *et al.*, 2016; Niveditha *et al.*, 2021) [4, 18]. The process flow diagram for the preparation of the defatted black soldier fly pre-pupae is given below in Fig.1.

Fig 1: Preparation of defatted black soldier fly pre-pupae insect flour

2.3 Development of Insect flour based bio-packaging films

The defatted insect flour was utilized for development of bio-packaging film. The defatted BSFP insect flour (4%) was dissolved in distilled water (100ml). The chitosan (1%) was prepared using 1% acetic acid. The constant amount of glycerol (0.5ml) was added as a plasticizer to promote flexibility of the bio-packaging film. The solvent-casting method was used to develop the insect based bio-packaging film. The filmogenic solutions were prepared using four different composition of insect flour with chitosan (0:100, 70:30, 50:50 and 30:70) which are represented as control, T1, T2 and T3. The control film was prepared without addition of BSFP flour. The solution was filtered and kept for overnight stirring to obtain homogeneity at 250 rpm. The filmogenic solutions were carefully poured on Teflon plates (150×100mm). The film was formed after being dryed at 25 to 27 °C for 48 hours. The obtained films were packed in zip-lock pouches and stored in dark place for further analytical testing purpose.

2.4 Morphology of defatted insect flour

The morphology of defatted insect flour (1g) was investigated using stereo microscope (Model: Leica S8 APO; Make: Leica microsystems, Wetzlar, Germany). The image is visualized under 1x magnification (Murat Kaya *et al*., 2016; Tiwari *et al*., 2021) [26].

2.5 Evaluation of insect flour based bio-packaging films

2.5.1 GSM of insect flour based bio-packaging films

The films were cut in constant size (20x20mm). The gram per square meter is calculated by measuring final dried weight of the insect flour based bio-packaging films (g) and divide by the value of surface area (mm) of the film (Setti L *et al*., 2020) [23]. The following equation was used to determine GSM.

$$\text{GSM (g/mm2)} = \frac{W}{A} \qquad (2.1)$$

Where, W is the film weight and A is the surface area of the insect flour based bio-packaging film

2.5.2 Evaluation ofcolor and a_w of insect flour based bio-packaging films

The color of BSFP film was determined using hunter lab calorimeter (Make: 45/0 LAV, Model: ColorFlex EZ, Germany). The L*, a*, b* values defines the lightness, redness and yellowness of the bio-packaging films. The color change, chroma and Hue angle was determined using the following formula for insect flour based bio-packaging film. The water activity reveals the amount of water present in the bio-packaging film, was measured using a_w meter (Thivya *et al*., 2021) [27].

$$\Delta E = \sqrt{(L - L^*)^2 + (a - a^*)^2 + (b - b^*)^2} \qquad (2.2)$$

$$\text{Chroma} = \sqrt{a^2 + b^2} \qquad (2.3)$$

$$\text{Hue angle } (\theta) = \tan^{-1}\frac{b}{a} \qquad (2.4)$$

Where, L, a, and b represent the color value of control and L*, a* and b* represent the color value of treatments

2.5.3 Evaluation of opacity

The opacity of bio-packaging films was determined using UV-Spectrophotometer (Model: UV-1800, Shimadzu, Japan). The obtained films were trimmed into 10× 30 mm and placed in the cell. The opacity was measured at 600nm wavelength. The following equation was used to determine the opacity of the bio-packaging films (Hosseini *et al*., 2014).

$$\text{Opacity } (\lambda/\text{mm}) = \frac{A_{600}}{\text{Thickness}} \qquad (2.5)$$

2.5.4 Evaluation of thickness and tensile strength

The films were cut into strips of 25 x 100mm (w×l) in size. The thickness was measured using screw gauge after being calibrated. The tensile strength was analyzed using texture analyzer (Make: TA HD Plus, UK, Model: Stable Micro Systems). The following equation was used to measure tensile strength (Jun Mei *et al*., 2013) [15].

$$\text{Tensile strength} = \frac{\text{Force (N)}}{\text{Surfacearea (mm}^2)} \qquad (2.6)$$

2.5.5 Antioxidant properties of film

The antioxidant properties were studied using DPPH assay. The films were cut in equal amount and added into 3ml of freshly prepared (0.1mM) DPPH solution which was prepared using methanol. The prepared solution was incubated in dark at room temperature for 60min. The absorbance was measure at 517nm against methanol using UV spectrophotometer (Thivya *et al*., 2021) [27].

$$\text{Antioxidant activity} = \left(\frac{\text{Acontrol}-\text{Asample}}{\text{Acontrol}}\right) \times 100 \qquad (2.7)$$

2.6 Statistical analysis

Each insect flour based bio-packaging film was analysed in triplicates. The insect flour based film were statistically analysed using one-way ANOVA and comparison post-hoc approach (Tukey) with a confidence level of 95% using the SPSS tool (ver.28; IBM, USA).

3. Result and Discussion

3.1 Insect flour yield percentage

The insect is initially pre-treated and freeze dried. The yield percentage of freeze dried insect flour after defatting using n-hexane was found to be 75.43 percent. The defatted insect is grinded and sieve, then flour was stored in -18C for further analysis.

3.2 Morphology of defatted Insect flour

The microscopic structure of defatted insect flour was investigates using stereo microscope. The magnification used for visualization was 1x. The defatted flour reveals the non uniform particle distribution. The size and shape of the particles were uneven. The particles were compact in structural integrity. The microscopic image (Fig. 2) reveals the color of the particles which are varied due to their chemical nature while undergoing pre-treatment and defatting process. The results were similar to the findings of Huang *et al*. (2019) [11] where the sample appears to be in compact in structure due to pre-treatment of BSFP sample.

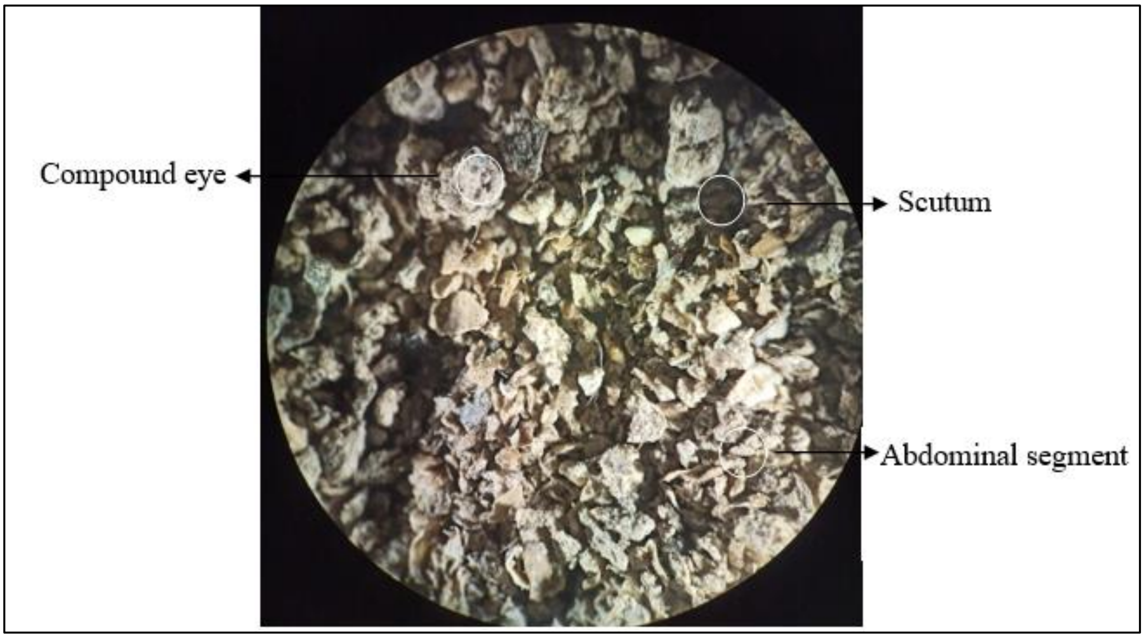

Fig 2: The visualization of defatted BSFP flour under microscopic view

3.3 Evaluation of insect flour based bio-packaging films

3.3.1 Evaluation of GSM

The gram per square meter of insect based bio-packaging film was determined. The GSM value gives the structural optimization by evaluating the thickness and density. The films were found to be significantly different with $p<0.05$. The GSM for the control film was found to be 50.81g/m^2 which depict the lightest among all other ratios (Table 1). The GSM of T1 was found to be 87.22 g/m^2 which have highest value due to the presence of higher ratio of insect flour along with chitosan (70:30). The GSM for T2 (50:50) and T3 (30:70) were found to be 54.40 and 43.69 g/m^{2}respectively which depicts to have decrease flour sample has the lightest weight. Similar results were obtained in Setti L *et al.*, 2020 [23] were the films were made only with BSFP protein.

3.3.2 Evaluation of color and a$_w$

The color of insect flour based bio-packaging films is given in the Table 1 and shown in Fig. 3. The control film was found to have (26.88) lesser lightness with l* value and negative a* indicates there is no red color influence in the control. The flour incorporated sample was observed to be slight darker color due to pigments present in the defatted flour. The b* value of T1 (11.39) which is very high represents the film to be darker brownish in color. The L*,a* and b* values of T2 and T3 samples shows to have less browning color due to the lesser amount on flour incorporated in the film production. In case of T2 and T3, the ratio was optimized as T2 (50:50) and T3 (30:70), the amount of insect flour was comparably reduced from T1. Thus the browning color was reduced in both the films. Similar color values were reported in Cheng, S. Y*et al.*, (2015) [6] in the film combination of zein protein and chitosan. The change in color (ΔE), Hue angle and chroma was computed (Table.1).The ΔE value of T2 and T3 were found to be no significant difference with $P<0.05$. The water activity of the films was found to have significant values in flour incorporated samples with $p<0.05$ due to the standard methodology (constant time-temperature) was used to develop the films. The water activity was computed and presented in Table 2. The films were found to have less than 0.70 percent water activity which generally resists microbial contamination.

Table 1: Color Evaluationof BSFP flour based bio-packaging films

Ratio Insect flour : chitosan	L*	a*	b*	ΔE	Hue angle	Chroma	GSM (g/m^2)
Control (0:100)	26.88±0.74^b	-0.49±0.08^d	1.75±0.32^d	-	73.33±0.55^c	1.85±0.26^b	50.81±0.40^c
T1 (70:30)	32.46±0.11^a	5.04±0.04^a	11.39±0.04^a	12.45±0.61^a	66.10±0.26^b	9.80±0.02^a	87.22±0.98^a
T2 (50:50)	25.57±0.13^b	0.22±0.02^c	4.37±0.03^b	3.75±0.46^b	78.23±0.58^{ab}	4.29±0.21^{ab}	54.40±0.18^b
T3 (30:70)	25.42±0.05^b	1.22±0.02^b	3.82±0.04^c	2.65±0.25^b	86.49±0.35^c	3.83±0.04^{ab}	43.69±0.53^d

Mean within rows with different superscripts are significantly different ($p<0.05$)

3.3.3 Evaluation of opacity

The opacity of insect flour based films was investigated using UV-spectrophotometer at wavelength of 600nm. The control films have more opacity with the value of 4.85λ/mm. The control film and T3 film were found to have no notable different with $p<0.05$. The T1 sample was found to have more opacity 10.08λ/mm followed by T2 and T3 sample were found 6.08 and 5.58λ/mm respectively. Also, T3 values were found to be significant with T2 value. Similar findings were reported by Tulamandi, S *et al.*, (2016) [29] in which the film was made from defatted soy protein.

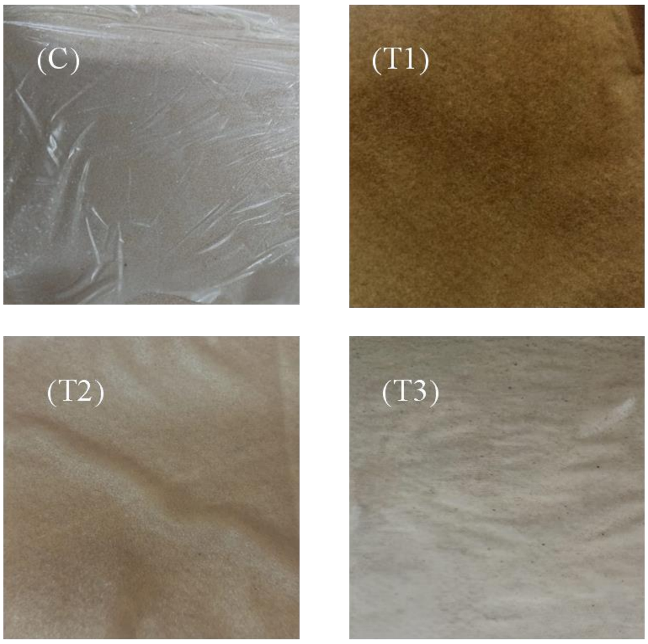

Fig 3: The developed BSFP flour based bio-packaging with different ratio of insect flour:chitosan C-control, T1-70:30, T2-50:50, and T3-30:70.

3.3.4 Mechanical properties of the insect flour based film

The mechanical properties were analyzed using texture analyzer and the values are depicted in Table 2. The thickness was measured at three different position of film using caliper thickness meter. The thickness of film was found to be increasing when the flour ratio is high. The film incorporated with insect flour was found to have no notable difference. The control film, T2 and T3 was found to have no significant difference with $p<0.05$. The addition of insect flour which has protein and chitin resulted structural integrity with greater extent. The insect flour and chitosan interaction of T3 was found to have optimistic range compared to other films. The control film and T3 film was found to have no notable difference with $p<0.05$. Also, T3 and T2 have found to be no significant difference. The results were accordance with the findings of Jun Mei *et al.*, (2013) [15]. The addition of glycerol as a plasticizer agent provides more flexibility to the obtained insect flour based bio-packaging films.

3.3.5 Antioxidant property

The antioxidant property results are the chitosan adding showed good antioxidant properties. The control film which has only chitosan and glycerol revealed higher antioxidant activity of 75.95 mg/ml when compared to other treatments. The antioxidant property for T1 was found to be 74.39 mg/ml. Similarly T2 and T3 film which has 73.74 and 73.91 mg/ml antioxidant property. The antioxidant values showed linear increase when the amount of chitosan incorporation increases. It is found that there is no significant difference with $p<0.05$ value between the T1, T2 and T3 films. The antioxidant values were in accordance with Cheng, S. Y*et al*., (2015) [6] reported in the film combination of chitosan incorporated with zein protein. Also, the results were in contradict to the findings of Chang *et al*., 2018 [5] due to different preparation method and incorporation of defatted BSFP flour which also have antioxidant property since melanin and ommochromes are present. Thus, obtained film has very optimistic antioxidant property which can be further used to prevent browning and oxidation in certain foods such as apples, pears and bananas.

Table 2: Properties of BSFP flour based bio-packaging films

Ratio Insect flour : chitosan	Water activity	Opacity (λ/mm)	Thickness (mm)	Tensile Strength (MPa)	Antioxidant activity (mg/ml)
Control (0:100)	0.60 ± 0.01^{b}	4.853 ± 0.40^{c}	0.07 ± 0.00^{b}	8.82 ± 0.22^{b}	75.95 ± 0.25^{a}
T1 (70:30)	0.64 ± 0.05^{a}	10.082 ± 0.22^{a}	0.12 ± 0.02^{a}	7.64 ± 0.31^{c}	74.39 ± 0.59^{b}
T2 (50:50)	0.65 ± 0.01^{a}	6.087 ± 0.48^{b}	0.09 ± 0.00^{ab}	9.58 ± 0.39^{ab}	73.74 ± 0.45^{b}
T3 (30:70)	0.65 ± 0.03^{a}	5.558 ± 0.44^{bc}	0.09 ± 0.00^{ab}	10.26 ± 0.25^{a}	73.91 ± 0.38^{b}

Mean within rows with different superscripts are significantly different ($p<0.05$)

4. Conclusion

The study explores by utilizing renewable insect resources to develop novel sustainable bio-packaging film. The black soldier fly prepupae were a promising source for producing sustainable films with high protein and chitin value. The insect reared in food waste was another advantage in food waste management. The BSFP based film has similar physical and mechanical properties of plastics. The mechanical property of insect flour incorporation provides linear increase, which shows a positive sign as insect flour can be used as reinforcement agent. The antioxidant property was found significantly high due to the presence of chitosan. The waste to wealth concepts by utilizing black soldier fly prepupae from food waste leads less frugal with optimistic protein and chitin compounds. The study reveals the suitability of insect flour with high nutritional and mechanical property in bio-packaging sectors. The bio-packaging films optimized from insect flour can be commercialized in RTE foods, confectionery, bakery industries.

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