Chapter 3

Optimization of the levels of mustard, soybean, and flaxseed meal for the development of biopolymeric films

3.1. Introduction

The oilseed production is one of the major industrial crops to produce oil all over the world (Wittkop et al., 2009). India comes forth in the production rate of oilseeds across the world after USA, China and Brazil, with average yield of 25 million tons annually (Reddy and Immanuelraj, 2017). Oilseeds are known for their fat providing dense food along with high amount of minerals, vitamins, fatty acids and specially fibres. Due to different geographic division and agro-ecological conditions in India, a large number of oilseeds are produced annually including edible, non-edible, combination of forest and horticulture variety and even from non-conventional sources. Groundnut, mustard-rapeseed, sunflower, nigar, sesame, soybean and safflower come under edible oilseeds. The popular linseed and castor oilseed are grouped under non-edible oilseeds. Coconut and palm oils are forest cum horticultural origin whereas rice bran, cotton seed and tobacco seed are considered under vegetable oilseeds (Jha et al., 2012).

The major by-product after oilseed processing is oilseed press cake. The term ‘cake’ and ‘meal’ refers to the two traditional methods of expelling the co-products using screw press or using solvents after extraction of oil from oilseeds respectively. However, there is uncertainty to use both terms (Arrutia et al., 2020). As per global waste treatment world records over 33% are openly dumped and only 5.5% are contributed in composting (Burke et al., 2018). Solid waste management issue is the biggest challenge to the authorities of both small and large cities’ in developing countries. Moreover, absence of understanding over different factors that affect the entire handling system of solid waste, a good alternative need is to be approached to reduce these problems.

Amongst the other oilseed meals available in market, Crambe cakes are reported to have largest traces of protein. They also contain a sizeable amount of nitrogen-free extracts, such as carbohydrates and fibers (Wanasundara, 2011). There are 35–40 % protein in canola seed cake and 31–36.7 % of protein in flaxseed cake (Teh et al., 2014). Flaxseed meals is by-product of flaxseed milling industries also has cellulose fibres along with proteins concentrated in them. The least processed and high protein of oilseed meal is soy flour. They are found to have 50% protein, 20% of dietary fibers, 20% of carbohydrates & 10% other components. Therefore, the use for the oilseed meals apart from food and feed needs to be treasured out (Arrutia et al., 2020). The use of these proteins for developing biopolymeric films material is an attractive alternative. Among available oilseed meals,

the mustard, flaxseed and soybean oilseed meals have been noted with high amount of phenolics, antioxidants and flavonoid compounds in them left along with high amount of proteins even after extraction of oils (Şahin and Elhussein, 2018). The produced products must use resources obtained from plants and must be industrially eco-efficient and sustainable for the future generations. The growing use of plant oils as sustainable feedstocks suggests that industrial oilseed meal might eventually serve as a source for oilseed meal-based biopolymeric films (Mohanty et al., 2005). For the usage of biopolymeric films and natural composite packaging materials with improved mechanical properties are demanded in a wider range of applications. Flaxseed meals have hydrophilic in nature and slightly branched structure. Mucilage or extract of flaxseed comprises of polysaccharide which has the capacity to ease in film forming traits (Waghmare et al., 2022). The films formed with flaxseed meals have high solubility thus, can also be healthy and eco-friendly giving us a good bio-packaging solutions (Bangar et al., 2021). Cellulose fibres from flaxseed meals can be used as reinforced composite films and to increase its strength, a compactable plasticizer or crosslinkers or different polysaccharides such as starch can be incorporated (Wu et al., 2013; Lee et al., 2015). The oilseed cakes had poor cohesive properties and needed to be blended with materials with higher cohesion (Gällstedt et al., 2017).

The lower level of microbial transglutaminase in the soy flour, significantly increases the tensile strength and surface hydrophobicity of soybean protein isolate films (Jiang et al., 2007). Preparation of film made individually from mustard seed meals without addition of any plasticizer or emulsifier delivers films with good tensile strength despite of its brittle texture in films (Hendrix et al., 2012).

Based on the above discussion, the current investigation focuses to overcome the issues of environmental pollution through aspects of utilize the potential of oilseed meals. Therefore, key objective of this work was to characterize the functional properties of oilseed cakes and formulate the biopolymeric films through blending of three different oilseed meals i.e., mustard seed meal, flaxseed meal and soybean seed meal and their synergistic effect investigation on water vapour permeability, tensile and elongation properties and solubility of the developed films and the films were further characterized on color, thermal and morphological properties. It can be helpful in exploring but also providing valuable information to small scale industrialists in utilizing by-products into vulnerable ones.

3.2. Materials and Methods

3.2.1. Sample Preparation

Mustard (*Brassica juncea* (TM-4)), Soybean (*Glycine max* (Pusa Soybean l)) and Flaxseed (*Linum usitatissimum* (T-397)) cakes were procured from local market of Tezpur, Assam, India. The oilseed cakes were selected based on the availability of oil producing industries nearby Tezpur. The sample were cleaned and screened manually by hand to remove unwanted husks and straws, then dried at 60˚C in tray drier (IKON, Delhi, India), grinded and sieved through a 45 µm mesh sieve, packed and stored in air-tight containers at 4˚C. The flour obtained after drying was defatted to decrease the oil content below 1% (Lalnunthari et al., 2019). The flour was defatted through heating the flour over Soxhlet apparatus with n-hexane at ratio (n-hexane: flour 30:1, v/w) and finally dried in tray drier (IKON, Delhi, India) at temperature 40 ºC for 24 h and stored in air-tight containers at 4 ºC for further analysis (Lalnunthari et al., 2019). n- hexane used in defatting of oilseed cakes and glycerol and soya lecithin used in film production were purchased from Merck Chemicals, India. All chemicals used in this study were of laboratory reagent grade. The oilseed cakes and meals of mustard seed, flaxseed and soybean seed are presented in Fig. 3.1.

Fig. 3.1. Oilseed cakes and defatted meals of mustard, flaxseed and soybean

3.2.2. Proximate analysis of oilseed cake

Oilseed cakes were analysed for moisture, protein, fat, fibre, ash and carbohydrate content according to Association of Official Analytical Chemists (Horowitz, 2005). The crude fat content of the sample was determined using n-hexane in Socs plus (SCS6, Pelican Equipment, Chennai, India) and the crude fibre with the help of Fibro plus (FES06, Pelican Equipment, Chennai, India). Protein content was estimated using micro-Kjeldahl method using Kel plus apparatus (Pelican Equipment, Chennai, India). Crude protein was estimated from total nitrogen using conversion factor of 6.25.

3.2.3. Functional properties of oilseed and defatted oilseed meals

3.2.3.1. Oil and Water absorption capacity

Oil and water absorption capacity of the oilseed cake were determined using method given by Omowaye-Taiwo et al. (2015) with slight modifications. Each 15 ml centrifuge tubes were pre-weighed and filled with 10 ml distilled water along with 1 g of sample was added. Then, centrifuge tubes were stirred for 5 min on vortex stirrer (Abdos, LabTech Pvt. Ltd., New Delhi) for uniform mixing. The suspension obtained was centrifuged using centrifuge (5430R, Eppendorf, Germany) for 20 min at 30˚C on 7000 rpm. The final supernatant was decanted into petri plates and volume was measured. Similar procedure was performed for oil absorption capacity by replacing water with oil. The difference between the initial oil/ water content used and the final decanted volume of supernatant gives the calculation of oil and water content absorbing capacity. The result was expressed in percentile considering the density of oil used (Eq. 3.1).

$$Oil/Water\ absorption\ capacity\ (\%) = \frac{initial\ oil/water\ content - final\ oil/water\ content}{initial\ oil/water\ content} \times 100 \quad (3.1)$$

3.2.3.2. Bulk density

The sample (5 g) was weighed into a pre-weighed measuring cylinder of 100 ml (W1). A new weight (W2) was recorded by tapping the measuring cylinder continuously until a constant weight was obtained. The obtained volume of the sample was recorded (V) and bulk density was calculated (Eq. 3.2) (Omowaye-Taiwo et al., 2015):

$$Bulk\ density\ (g/ml) = \frac{W2 - W1}{V} \quad (3.2)$$

3.2.3.3. Foam capacity and Foam stability

The method of foam capacity (FC) and foam stability (FS) of oilseed cake was carried out. Distilled water (100 ml) with 0.5 g of sample were dispersed in a 250 ml beaker. The solution was stirred on magnetic stirrer (Remi Bharat Scientific World, Karnataka, India) at speed of 1500 rpm for 5 min and immediately transferred to 250 ml graduated cylinder and readings were recorded for foam capacity whereas, the foam stability was calculated by the remaining volume after every 10 min for 1 h. All the readings were taken in triplicate. The formulae for calculating foam capacity and stability are given in Eq. 3.3 and 3.4 (Omowaye-Taiwo et al., 2015).

$$FC(\%) = \frac{volume\ after\ homogenisation - volume\ before\ homogenisation}{volume\ before\ homogenisation} \times 100 \quad (3.3)$$

$$FS(\%) = \frac{volume\ of\ foam\ after\ certain\ time\ interval}{initial\ foam\ volume} \times 100 \quad (3.4)$$

3.2.3.4. Emulsion capacity and stability

Emulsion capacity (EC) and emulsion stability (ES) was determined in reference to method suggested by Omowaye-Taiwo et al. (2015) with modifications. Oilseed cake flour of 0.5% m/v concentration was dispersed in deionized water and mixed continuously for 20 min at 500 rpm at magnetic stirrer (Remi, Bharat Scientific World, Karnataka, India) 30 ml of this solution was mixed with 10 ml of soybean refined oil and homogenized at 2000 rpm to form an emulsion. After 1 min of the homogenizing the aliquot volume of the emulsion was measured and emulsion capacity was determined (Eq. 3.5).

$$EC\ (\%) = \frac{Height\ of\ emulsifier\ layer}{Height\ of\ the\ contents\ in\ the\ tube} \times 100 \quad (3.5)$$

The emulsion stability was determined using method reported by Iyenagbe et al. (2017). The graduated cylinder was heated with above formed emulsion into 80˚C water for 30 min. The formulae for calculating emulsion stability are expressed in Eq. 3.6.

$$ES\ (\%) = \frac{Height\ of\ remained\ emulsion\ layer}{Height\ of\ initial\ emusion\ layer} \times 100 \quad (3.6)$$

3.2.3.5. Least gelation concentration

Each 10 ml test tubes were filled with suspension having sample concentration of 2–20% (m/v) prepared with distilled water (Sathe et al., 1982). Then, the test tubes were allowed to heat for at least 1 h in boiling water bath water bath (Riviera Glass Pvt. Ltd., Mumbai, India) further cooled in running tap water and followed by cooling in refrigerator at 4˚C for 2 h. The least gelation concentration was determined by the one from which suspension do not fall when inverted through the test tube.

3.2.3.6. Colour analysis

The colour of the oilseed cakes and defatted meals were measured using Hunter Lab colorimeter (Ultrascan VIS, Hunter Lab. Inc., USA) that absorbed the range on CIE Lab scale (L^*, a^* and b^*). White and black standard tiles were used to calibrate the instrument. The colour scale was determined over lightness terms as L^* ($L^* = 0$ for black and $L^* = 100$ for white) and chromaticity parameter as 'a^* and b^*' as '($-a$) greenness; ($+a$) redness and ($-b$) blueness; ($+b$) yellowness' respectively as suggested to the reference described in Teh et al. (2014).

3.2.4. Statistical analysis

Each analysis was performed in triplicates and data were reported as mean ± SD (standard deviation). One-way ANOVA was used to determine the critical difference of means and variance amongst the dissimilar samples. Duncan test with equal variances was performed at a significance level of $P \leq 0.05$ using IBM SPSS version 2.3 statistics software.

3.3. Blended film preparation

The film was prepared through casting method as described by Hendrix et al., (2012). Twenty film suspension named (A to T) was formulated as per response surface methodology mixture design prediction. The oilseed meal mixture was taken constant at 6g w/v. The blended oilseed meals ranged (1-100%) were added with glycerol (75% w/w) as plasticiser and soy lecithin (2% w/w) as emulsifier in 100 ml distilled water along with constant stirring on magnetic stirrer (ABDOS MS H280 Pro, India) for uniform mixing at temperature of 50 ºC for 15 min. The suspension was also heat treated in hot water bath (Modern, New Delhi) at 90 ºC for 30 min with stirring and cooled in iced water. The air bubbles were removed by keeping the beaker with suspension stable for 10 min and stirred

slowly thereafter before casting into 150×25 mm petri dishes and dried at 65 ºC for 72 h to produce a smooth and even films. After drying, the films were peeled off and stored in zip lock pouches before placing in desiccator filled with dried self-indicating crystal silica gel (RH 0%) and kept at room temperature for further analysis.

Table 3.1. Proportion of the components (oilseed meals) for development of biopolymeric films

Runs	Mustard seed meal (X1)	Flaxseed meal (X2)	Soybean seed meal (X3)
1	100.000	0.000	0.000
2	66.631	16.555	16.814
3	50.001	49.999	0.000
4	50.001	49.999	0.000
5	0.000	0.000	100.000
6	100.000	0.000	0.000
7	50.001	0.000	49.999
8	0.000	100.000	0.000
9	16.982	66.344	16.674
10	33.613	33.182	33.205
11	0.000	16.848	83.152
12	0.000	0.000	100.000
13	50.001	0.000	49.999
14	17.048	16.581	66.371
15	0.000	49.999	50.001
16	0.000	100.000	0.000
17	0.000	50.000	50.000
18	50.000	25.000	25.000
19	25.000	50.000	25.000
20	25.000	25.000	50.000

Design: D-optimal Mixture design
Independent Variables: Mustard seed meal, X1:(1-100%), Flaxseed meal, X2: (1-100%) and Soybean seed meal, X3: (1-100%)

3.3.1. Experimental design

The mixture design is a design which uses different proportions of independent factors to form a blend, where the sum of different factors must be 100% according to Sachs, (2012). The mixture design for 3 variables can be equated as in equation (3.7).

$$\sum_{i=1}^{q} xi = x1 + x2 + \cdots + xq = 1 \quad (3.7)$$

Where, xi represents the proportion of i^{th} component in the mixture.

A D-optimal mixture design was used to study the effects of interactions between the ingredients of film development as well as on the barrier, mechanical and physical performance of the films. The mixture design was done using Design Expert Software version 7.0.0 (Statease Inc.) with 20 experimental runs (including 5 replicates) are presented in Table 3.1. The proportion of glycerol and soy lecithin ratio to be used in film formation was fixed through preliminary trials to obtain a film which is neither sticky nor rigid in touch. The limit of each constraint (oilseeds) for film production were pre-determined through preliminary trials by evaluating the developed films in account of flexibility, homogeneity, and stickiness.

The minimum and the maximum limits of each mixture constraint were as follows: Mustard seed meal, X1:(1-100%), Flaxseed meal, X2: (1-100%) and Soybean seed meal, X3: (1-100%) were taken as independent variables. The measuring constraints as dependent variables were taken as function of responses (Y) properties in terms as water vapor permeability (Y1), water solubility (Y2), swelling property (Y3) and tensile strength (Y4) for the development of best biopolymeric film.

The pseudo-components were calculated using Eq. (3.8).

$$Ps_x = C_x\text{-}a_y/\Sigma a_y \quad (3.8)$$

Where, Ps_x is the pseudo-component of each component, C_x is the real concentration of the component, a_y is the lower limit of the real component, and Σa_y is the sum of the lower limits of the three components in the mixture design. Table 3.1 shows the real components and the pseudo-components of each mixture (Zanela et al., 2015).

3.3.2. Characterization of blended films

3.3.2.1. Film thickness and moisture content

The thickness of the film was determined using manual micrometer (Alton M820-25, China) having sensitivity of 0.01 mm. The thickness of six different films were taken and the average thickness of the film was calculated (Pelissari et al., 2012). The moisture content was determined by calculating the amount of initial weight lost in drying the sample into hot air oven at 105°C for 24 h (Horowitz, 2005).

3.3.2.2. Solubility

Film specimen was cut into 20×20 mm and dried into hot air over for 105°C for 24 h until constant weight was gained. After drying the films were submerged into 40 ml distilled water filled in 50 ml glass beaker with occasional agitation for 24 h at room temperature. The leftover solid part not solubilized in water were carefully removed from glass beaker and kept for drying again at 105°C for 24 h into hot air oven until constant weight is obtained. The total soluble matter (% TSM) of film were calculated using method described in Ojagh et al., (2010) as in equation 3.9.

$$TSM\ (\%) = \frac{initial\ dry\ weight - final\ dry\ weight}{initial\ dry\ weight} \times 100 \quad (3.9)$$

3.3.2.3. Water vapor permeability

The film was cut into 20×20 mm, fixed and sealed on glass beaker with round edges of diameter 50 mm partially filled with dried $CaSO_4$ powder (0% Relative humidity). The glass beaker was initially weighed and placed in desiccator filled with saturated K_2SO_4 solution of relative humidity of 97% and placed undisturbed in incubator set at 25 ºC. The deviation of weight of film due to mass exchange taken place in desiccator is recorded for 7 days in every 24 h for the calculation of water vapour transmission rate (WVTR). The slope was calculated on weight change with function of time as linear regression (weight change vs time) and slope (g/h) divided by the film area given the value of water vapour transmission rate (Wu et al., 2013). Water vapour permeability was calculated using equation 3.10.

$$WVP\ (g/Pa\ h\ m) = \frac{WVTR}{P(R1-R2)} \times X \quad (3.10)$$

Where, P = saturated vapor pressures of water (Pa) at room temperature

R1= relative humidity of the desiccator with K_2SO_4 solution

R2= relative humidity of the cup with dried $CaSO_4$ powder

X = thickness of film (m) and the driving force in experimental conditions were taken as [P (R1 – R2)] is 3073.93 Pa.

3.3.2.4. Mechanical properties

The textural and elongation property was determined as per literature of research work by Zavareze et al., (2014) using texture analyser (TA-HD Plus Stable microsystems, UK). The texture analyser tension mode was pre-set at pre-test speed, test speed, post-test speed as 5 mm/s, 1 mm/s, 5 mm/s respectively. The biopolymeric film was cut into 60×20 mm size pieces for measurement and placed with probe load weighing 5 kg. the film was stretched upward until fallout/rupture. Each sample was run in triplicate. After the experiment, the maximum force and the distance of the grips on curves was recorded for further calculation of the tensile strength and the elongation at break. The equation for the calculation of the mechanical properties were as given in equation (3.11) & (3.12):

$$Tensile\ strength\ (MPa) = \frac{Maximum\ force\ of\ the\ film\ (N)}{Cross\ sectional\ area\ of\ the\ film\ (m2)} \quad (3.11)$$

$$Elongation\ at\ break\ (\%) = \frac{Distance\ of\ rupture\ of\ film}{Onset\ distance\ of\ the\ separation\ of\ film} \times 100 \quad (3.12)$$

3.3.2.5. Colour analysis

The colour analysis of the biopolymeric films were done through the Hunters Calorimeter (Ultra scan VIS; Hunter lab, USA). The values were recorded in terms of L*, a* and b*. The calorimeter was calibrated by white and black standard tiles having L= 96.84, a= -0.20 and b= 2.01. The scale of colour was determined as L* (L*=0 for black and L*=100 for white) and the parameters of chromaticity as 'a* and b*' as '(–a) greenness; (+a) redness and (–b) blueness; (+b) yellowness' respectively. The whiteness index (WI) was measured using Eq. (3.13) as presented in study of Lee et al. (2016).

$$WI = 100- [(100-L)^2 + a^2 + b^2]^{0.5} \quad (3.13)$$

3.3.2.6. Scanning electron microscopy (SEM)

The SEM is used to study the surface morphology of the sample. The images of the biopolymeric films were produced through a Scanning Electron Microscope model JSM 6390LV (JEOL, Japan). The films were priorly dried at 60˚C in hot air oven for 5 h and then the films were cut and finely coated with gold deposition by means of a plasma sputtering apparatus for analysis both for surface and cross-section study (Deepa et al., 2016).

3.3.2.7. Differential scanning calorimetry (DSC)

The thermal properties of the biopolymeric films were studied through Differential Scanning Calorimetry (214, Polyma, NETZSCH, Germany). 10 mg of the sample was hermetically sealed in an Aluminium pan and placed in DSC chamber. The film sample was heating at temperature 20ºC to 300ºC at the rate of 10.0K/min. The determination of the glass transition temperature (T_g), peak temperature (T_p), onset temperature (T_o), and denaturation enthalpy (ΔH) of biopolymeric films were recorded (Zhang et al., 2018).

3.3.2.8. Fourier-transform infrared spectroscopy (FTIR) analysis

The FTIR of the biopolymeric films were analysed using machine Bruker Equinox 55 spectrometer (Bruker Banner Lane, Conventry, Germany). The films were grinded into fine powder and mixed with IR grade KBr to form pallets using hand operated pressing machine at roughly 12,000 psi pressure. The film sample was analysed in the region between 400 to 4000 cm^{-1}. The data was collected and graph obtained was analysed in Origin Pro 9.0 software. The graph was properly attenuated of total reflectance, baseline corrected and normalization of spectra was done (Singha et al., 2023a).

3.3.2.9. Statistical analysis

The Software Design Expert version 7.0.0 (Statease Inc.) was used to achieve an experimental design for blending of the oilseed meals to produce biopolymeric films and to predict the effect of dependent variables through mathematical models and 2D surface contour graphs. Further to analyse the rationality and relativity, the Scheffé model was used, one way ANOVA (analysis of variance) was done to compare the means of the results obtained by significance of $p<0.05$.

The data extracted were calculated using the special cubic model equation for the three components as shown in equation (3.14).

$$Y = \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{12}X_1X_2 - \beta_{13}X_1X_3 - \beta_{23}X_2X_3 - \beta_{123}X_1X_2X_3 + \gamma_1X_1X_2(X_1-X_2) - \gamma_2X_1X_3(X_1-X_3) + \gamma_3X_2X_3(X_2-X_3) \quad (3.14)$$

Where, Y= responses of the method, β_s and γ_s = parameters of the linear and crosslinked product of the model and X1, X2 & X3= pseudo components of mustard, flaxseed, and soybean seed meals respectively. The positive and negative values in

equations for binary coefficients β_{ij} and γ_{ij} indicates synergistic effects and antagonism respectively.

3.4. Results and discussion

(a) Characterization of the oilseed and defatted oilseed meals

3.4.1. Proximate composition of oilseed cakes

The moisture content, crude protein, total ash, crude fibre and crude fat are presented in Table 3.2. Moisture content is an important factor for storage of products for longer period of stability. The moisture content of mustard seed, flaxseed and soybean seed cakes were reported as 10.55 ± 0.49%, 9.95 ± 0.05% and 9.6 ± 0.52% respectively which were less enough to preserve the floured samples. The moisture con- tent level below 12% were considered safe for storage of materials (Yılmaz et al., 2017). Similar findings were reported for flaxseed cake and defatted flaxseed meal with moisture content of 7.94 and 9.37% respectively (Tirgar et al., 2017). The protein content of mustard seed, flaxseed and soy- bean seed cakes were found as 31.06 ± 0.61%, 32.74 ± 0.53% and 44.46 ± 2.95% respectively. Protein plays an important role while estimating nutritional and feed value. In oilseed cakes, the value differs in terms of its initial seed composition and oil yield during pressing in the available environment (Yılmaz et al., 2017). This high amount of proteins portrays its potency in various product development sectors including human consumption in healthy way. The results are in line with protein content of defatted canola meal protein, mustard seed cake, flaxseed cake and defatted meal (Klockeman et al., 1997; Sehwag and Das et al., 2015; Tirgar et al., 2017). They reported 33–40% protein content in mustard seed cake. Ash content in any sample shows the amount of minerals content per gram of the sample. The ash content was found to be 8.95 ± 1.06%, 9.45 ± 0.07% and 8.6 ± 0.6% in mustard seed, flaxseed and soybean seed cake respectively (Table 3.1.). Devi and Devi, (2011) reported the 8% ash in mustard seed cake. Mustard seed, flaxseed and soybean seed cake were observed as 15.8 ± 0.26%, 66 ± 0.5% and 45.83 ± 0.76% crude fibre respectively. The results of proximate indicated that mustard, flaxseed and soybean seed meal flour contained 7.26 ± 0.16%, 4.77 ± 0.35% and 1.20 ± 0.42% of crude fat respectively. The fat content was reported as 5.35 ± 0.02% in partially defatted non-roasted flaxseed flour (Hussain et al., 2008). Tirgar et al., (2017) reported flaxseed cake and flaxseed meal had 5.26 & 5.72% ash, 12.02 & 1.87% fat and 44.05 & 48.90% of carbohydrate respectively. Yılmaz et al. (2017) observed that even after cold pressing procedure in oil extraction the fat content remains were found in cake of Capia pepper

oilseeds. This can be due to heat-cold degrading conditions while extraction. Thus, oil remains in cakes can be acceptable. Remains of oil content in the sample predict the presence of nutritional value (energy value) and oxidative stability of future product development. Solvent extraction can reduce the oil content below 1%. The carbohydrate content in the oilseed cakes were found as 39.65 ± 0.7%, 43.89 ± 0.18% and 30.2 ± 2.4% in mustard seed, flaxseed and soybean seed cakes respectively. Gutiérrez et al. (2010) reported 48.7% non- nitrogenated extract in flaxseed cakes.

Table 3.2. Proximate composition of different oilseed cakes

Parameters (%)	Mustard seed cake	Flaxseed cake	Soybean seed cake
Moisture content	10.55±0.50^{b}	9.96±0.05^{ab}	9.6±0.53^{a}
Crude protein	31.06±0.61^{a}	32.74±0.53^{a}	44.47±2.95^{b}
Total ash	8.95±1.06^{b}	9.45±0.07^{c}	7.53±0.15^{a}
Crude fiber	15.8±0.26^{a}	66±0.5^{c}	45.83±0.76^{b}
Crude fat	7.3±0.17^{c}	4.78±0.36^{b}	1.20±0.42^{a}
Carbohydrate (by difference)	39.65±0.7^{b}	43.89±0.18^{c}	30.2±2.4^{a}

The values of different properties are expressed as mean ± standard deviation.

3.4.2. Functional Properties

Different oilseeds cakes have differences in their functional properties which make them unique. Since most of them are found useless after extraction of oils from them, a part of cattle feeding, all the rest are dumped. The process of defatting reduced the oil content of all the oilseed cakes up to 0.48% in mustard seed, 0.21% in flaxseed and 0.15% in soybean cakes. Decrease in fat content increases the probability of development of new products to improve commercial feasibility.

3.4.2.1. Water and Oil Absorption Capacity of Oilseed Cakes and Meals

Table 3.3 shows comparison between oilseed cakes and defatted oilseed meal on the basis of oil and water absorption capacity. The absorption capacity of water or oil is associated to the ability of absorption in limited water supply (Oladele and Aina, 2007). The highest water absorption capacity was found in defatted soybean seed meal. The range of

water absorption capacity was 10 to 18.33% in oilseed cakes and 13.33 to 24.67% in defatted oilseed meal. Defatting the meals with thermal treatments increases the water absorbing capacity (Moure et al., 2006). The values were found higher than other reported oilseed cakes. The water absorption capacity of defatted conophor nuts and raw conophur flours were 1.18% and 1.36% respectively (Iyenagbe et al., 2017). Amza et al. (2011) reported water absorption capacity in defatted ginger bread plum seed meal and defatted peanut meal was 3.01% and 2.96% respectively. But in few literatures, the water the absorption capacity was found higher. WAC for *Cucumeropsis mannii* raw dried defatted seed flours were 216.67–267.67% (Omowaye-Taiwo et al., 2015), and for groundnut seed cake were 303.33 and 306.67 ml H_2O/100 g (Fekria et al., 2012). Such large difference might be due to different structures of proteins in different samples and high hydrophilic carbohydrates constituents responsible for variation in the WAC of the flours. There were limited reports related to functional properties of mustard, flaxseed and soybean seed cakes and meals. Water absorption capacity is an important property in the preparation of processed food such as bakery, sauces, soups and meat products or such similar products and beneficial for quality and yield purpose (Yılmaz et al., 2017). The highest oil absorption capacity with 14.33% was found in flaxseed cake. The oil absorption capacity values of oilseed cake and defatted oilseed meals were 1 to 14.33% and 10 to 13.11% respectively. The values of OAC were found higher in comparison with raw defatted conophor nut flours, defatted gingerbread plum seed meal and defatted peanut meal having OAC was 1%, 3.12% and 3.11% respectively (Amza et al., 2011; Iyenagbe et al., 2017). Whereas, OAC was higher in *Cucumeropsis mannii* raw dried defatted seed flours with 292.00–345.00% (Omowaye-Taiwo et al., 2015) and 293.33 and 286.67 ml oil/100 g in defatted ground- nut seed cake (Fekria et al., 2012). Hence mustard, flaxseed and soybean oilseed cakes are considered as moderate OAC. The lower values of oil absorption shows that they may have lower hydrophilic protein and lower binding to lipids with lower retention ability (Kinsella and Melachouris, 1976). Oil absorption capacity is an approved property in meat production emulsification (Foegeding and Davis, 2011).

3.4.2.2. Bulk Density

Bulk densities of oilseed cakes and defatted oilseed meal are presented in Table 3.3. Bulk density is a property of measuring the heaviness of the flour sample and depends on particle size intermolecular forces and number of positions in connection (Peleg, 1983). Bulk density is required in food industry for packaging, flour handling and in wet process

handling (Amza et al., 2011). The result indicated that defatted soybean seed meal had the highest value of bulk density. The values of bulk densities in oilseed cakes ranged from 0.40 to 0.51 g/ml whereas 0.48 to 0.54 g/ml in defatted oilseed meals. The result obtained showed an increase in bulk density after the defatting of oil- seed cake. The findings are in line with the bulk density of soybean flour (0.47 g/ml)(Chau and Cheung, 1998), whole and defatted pumpkin seed flour (0.57 and 0.37 g/ml)(Rodríguez-Miranda et al., 2012). A reported values are higher compared to the reported bulk density of the defatted gingerbread plum seed meal (0.30 g/ml)(Amza et al., 2011) and lower than defatted groundnut seed cake (0.71 g/ml)(Fekria et al., 2012).

3.4.2.3. Foam Capacity and Foam Stability of Oilseed Meal

Foaming capacity was observed highest in soybean seed cake with 21.19 ± 0.67%. The foaming capacity of oilseed cakes ranged from 3.10 to 21.19% and 14.69 to 17% in case of defatted oilseed meal (Table 3.3). It is observed that there was decrease in foaming capacity in soybean seed cake after defatting of oilseed cake. Lowering in foaming capacity may be due to lower protein content in defatted oilseed meal since foaming is always related to the amount of solubilized protein (Narayana and Narasinga Rao, 1982) and the amount of polar and non-polar lipids in a sample (Nwokolo, 1987). Figure 3.2 shows the foaming stability of oilseed cakes and defatted meals. Defatted soybean meal showed highest foaming stability. Chau and Cheung, (1998) found defatted soybean seed flour had highest foaming stability than other leguminous flours standing for 5 to 120 min. Sharma et al. (2010) measured different oilseeds meals of almond, chestnut, hazelnut, pine nut, brazil nut, pistachio nut and soybeans W82 variety and found that the foam capacity and stability were above 40% and were independent to their source material. The foaming capacity was reported lower in defatted groundnut seed cakes (4.2 and 4.0 ml/100 ml water) and high foam stability was recorded with only 4% of the foam loses up to 120 min (Fekria et al., 2012).

Table 3.3. Water and oil absorption capacity, bulk density and foaming capacity of oilseeds cakes and meals

Samples	Water absorption capacity (%)	Oil absorption capacity (%)	Bulk density (g/ml)	Foaming Capacity (%)
Mustard seed cake	11.67±1.70^{a}	1.00±0.82^{a}	0.40±0.01^{a}	3.10±0.12^{a}
Defatted mustard seed meal	18.89±1.92^{b}	12.44±1.54^{b}	0.48±0.02^{bc}	17.00±0.29^{d}
Flaxseed cake	18.33±2.36^{b}	14.33±4.03^{b}	0.46±0.03^{b}	11.95±0.09^{b}
Defatted flaxseed meal	13.33±3.33^{a}	10.00±0.67^{b}	0.50±0.01^{cd}	16.34±0.50^{d}
Soybean seed cake	10.00±0.82^{a}	4.33±4.03^{a}	0.51±0.01^{cd}	21.19±0.67^{e}
Defatted soybean seed meal	24.67±1.76^{c}	13.11±1.02^{b}	0.54±0.02^{e}	14.69±0.76^{c}

The values of different properties are expressed as mean ± standard deviation. Mean followed by different letters (a, b, c, d, e) in the same column differs significantly ($p \leq 0.05$).

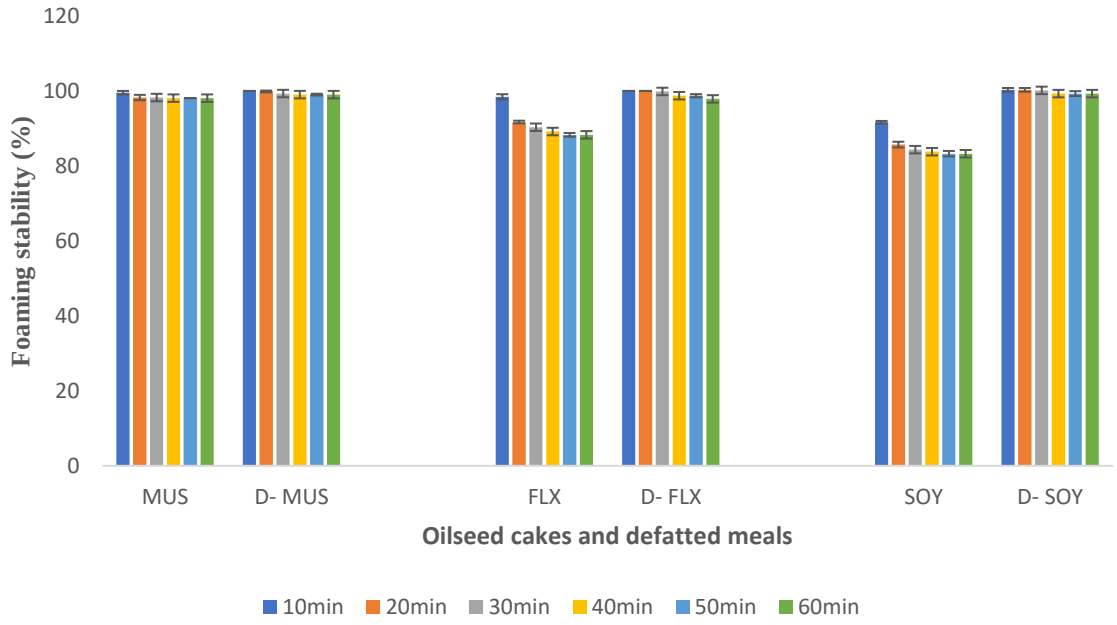


Fig. 3.2. Foaming stability of different oilseed cakes and defatted meals (D- defatted, MUS- mustard meal, FLX- flaxseed meal, SOY- soybean meal)

3.4.2.4. Emulsion Capacity and Stability

The property of mixing two immiscible liquids (water and oil) is known as emulsion capacity (Amza et al., 2011). Emulsion capacity was found highest in defatted soybean meal. The emulsion capacity was found in range of 40.69 to 51.38% in oilseed cakes whereas in defatted meals the range was 47.26 to 57.18% (Table 3.4). Similar report was observed in whole pumpkin seed meal and defatted pumpkin seed meal with emulsion capacity of 54.01 and 61.71% respectively (Rodríguez-Miranda et al., 2012). Increment in values can be due to protein unfolding increase emulsification activities and enhancement of hydrophobic domain and developing flexibility (Moure et al., 2006). Higher is the protein content in the sample, better is the emulsifying capacity of the sample. Emulsion stability is the measurement of volume of water separation from emulsion in few hours of interval (Oshodi et al., 1999). Emulsion stability was seen highest in flaxseed meal. Madhusudhan and Singh (1985) reported reduction in emulsion and foaming capacities in flaxseed flour. The values of emulsion capacity and stability varied can be due to the different measurement techniques in studies and material sources from various location (Yılmaz et al., 2017). Proteins are formed of charged and uncharged amino acids which possibly helps in formation of emulsion and their polarity and non-polarity determines the hydrophilic and hydrophobic property which makes a surfactant stable. Hence, decrease in protein decreases the emulsion stability (Ulloa et al., 2011).

3.4.2.5. Least Gelation Concentration

Gelation property is the ability of forming gel in the flour during any treatments and are also nature dependent over the presence of proteins and non-proteins (starch, gums etc.) elements present in the flour sample. As shown in Table 3.4, least gelation concentration attained similar results in all oilseed cakes, defatted meal of mustard seed and soybean seed cakes (i.e., 18%), whereas it is less in case of flaxseed meal (8%). There was a significant decrease in least gelation capacity in defatted flaxseed meal in comparison to flaxseed cake. Similar results were found for soybean seed flour with 17% (Chau and Cheung, 1998) and cashew nut with 10% LGC (Omowaye-Taiwo et al., 2015). A study on least gelation concentration reported by Sharma et al. (2010) on almonds, chestnuts and Brazil nuts resulted 6%, 8% and 8% respectively whereas soybean concentration was reported as 16% LGC. Even Hrckova et al. (2002) also measured LGC with similar results on commercial defatted soy flour and found 2% with gel sliding on test tube wall shows gelling property.

Table 3.4. Emulsion capacity, stability and least gelation concentration of oilseeds cakes and meals

Samples	Emulsion capacity (%)	Emulsion stability (%)	Least gelation concentration (%)
Mustard seed cake	40.69±0.95[a]	118.23±1.91[b]	18±0.0[b]
Defatted mustard seed meal	47.26±2.18[b]	111.04±2.82[a]	18±0.0[b]
Flaxseed cake	51.38±0.14[c]	128.46±3.51[c]	18±0.0[b]
Defatted flaxseed meal	55.86±2.02[d]	108.15±1.30[a]	8±0.0[a]
Soybean seed cake	49.98±1.88b[c]	107.52±2.23[a]	18±0.0[b]
Defatted soybean seed meal	57.18±2.47[d]	119.89±2.80[b]	18±0.0[b]

The values of different properties are expressed as mean ± standard deviation. Mean followed by different letters (a, b, c, d, e) in the same column differs significantly ($p \leq 0.05$).

3.4.2.6. Colour Measurement

The colour measurement of the oilseed cakes and meals are presented in Table 3.5. The result indicate that defatted soybean seed meal has the highest L^* value (81.54 ± 0.17) in comparison to mustard and flaxseed cakes and meals. While a^* value was recorded highest in mustard seed cake (5.39 ± 0.36) and lowest in case of defatted soybean seed meal (2.18 ± 0.29). The b^* value was found comparatively more in soybean seed cake and defatted meal compared with cakes and meals of mustard seed and flaxseed. There was an increase in the L^* values and decrease in the a^* and b^* values recorded in all the oilseed meals after defatting. This may be due to removal of oil from oilseeds which may impart dark colour to oilseed cake. Similar trend of colour values was observed in case of whole pumpkin seed meal and defatted pumpkin seed meals (Rodríguez-Miranda et al., 2012).

Table 3.5. Colour measurement of oilseeds cakes and meals

Samples	L*	a*	b*
Mustard seed cake	55.28±2.65[a]	5.39±0.36[c]	12.84±1.97[b]
Defatted mustard seed meal	61.66±1.16[c]	4.12±0.25[b]	12.88±1.89[b]
Flaxseed cake	58.99±0.53[b]	4.48±0.20[b]	9.80±0.44[a]
Defatted flaxseed meal	59.26±1.46[b,c]	4.23±0.24[b]	9.12±1.22[a]
Soybean seed cake	80.96±0.59[d]	2.36±0.08[a]	18.62±0.42[c]
Defatted soybean seed meal	81.54±0.17[d]	2.18±0.29[a]	18.06±1.30[c]

The values of different properties are expressed as mean ± standard deviation. Mean followed by different letters (a, b, c, d) in the same column differs significantly ($p \leq 0.05$).

3.5. Results and discussion

(b) Study of optimized oilseed meals blended films

3.5.1. Experimental modelling of Mixture Design

The selected conditions for the development of all the biopolymeric films were done in correspondence to the mixture design to develop a bubble free smooth surfaced and homogeneous films based on oilseed meals. As per the experimental trails, it was found that the individual oilseed meals were unable to provide uniform films with glycerol and the formulated films were found brittle with cracks. Similar texture was found in rapeseed flour and rapeseed cake residuals after compression moulding developed in Johansson et al., (2012). This texture in films may be due to absence of emulsion forming property in suspensions by the oilseed meals for the film production. The limitations were overcome through blending the different oilseeds meals (mustard, flaxseed, and soybean seed meals) with the help of mixture design along soy lecithin and glycerol (pre-determined amount in trails). The preparation of suspension of each constraint along with its pseudo constraints and the results are presented in Table 3.6.

To analyse the accuracy of the fitted model used for the development of the films, different components of ANOVA such as lack of fit, sum of square (PRESS), coefficient of variance (R^2), p-value, Prob> F etc. are listed in Table 3.7. The contour plot of oilseed meals as a function of solubility is presented in Fig. 3.3 (a). The results showed that with the addition of soybean seed meal in the composition of the film formation, the solubility increases. The contour plot of biopolymeric film as a function of tensile strength are shown in Fig. 3.3 (b). The results showed that the biopolymeric film with highest percentage of mustard seed meal, had greater tensile strength. But, the results of elongation at break on contour plot shown in Fig. 3.3 (c) shows that the biopolymeric film with highest amount of mustard seed meal, had lowest elongation at break since the elongation at break property is inversely proportional to tensile property. The contour plot in Fig. 3.3 (d) explains the water vapour permeability of the biopolymeric films. The results of water vapour permeability highly depend on the thickness of the films. The film with highest thickness may have lowest water vapour permeability. The optimized conditions used for the development of a successful biopolymeric film includes minimum solubility, minimum water vapour permeability, in range of tensile strength and elongation at break. The results shown in the Fig. 3.3 reviled that the higher amount of flaxseed meal played an important role in the development of biopolymeric films.

3.5.2. Experiential model prediction on biopolymeric film properties

The Mixture Component Coding is also known as L_Pseudo. The equations (3.15, 3.16, 3.17 & 3.18) are the L_Pseudo components of the final equations of different properties of the biopolymeric films obtained from ANOVA. These equations indicate the empirical models produced by fitting the responses on mixture design. In order to study the process and response variables obtained from mathematical model and the constructed 2D contour plots between dependent and independent variables.

Final Equation in Terms of L_Pseudo Components:

Percent Solubility = 55.09 X1+ 53.51 X2+ 60.29 X3+ 42.94 X1X2- 21.87 X1X3- 3.78 X2X3- 109.86 X1X2X3+ 422.61 X1X2 (X1-X2)- 168.98 X1X3 (X1-X3) + 38.51 X2X3 (X2-X3) (3.15)

Tensile Strength = 12.88 X1+ 12.11 X2+21.23 X3- 1.01 X1X2- 29.89 X1X3- 41.78 X2X3+ 666.98 X1X2X3+ 295.09 X1X2 (X1-X2)- 76.46 X1X3 (X1-X3)+ 116.19 X2X3 (X2-X3) (3.16)

Percent Elongation = 18.45 X1+ 14.56 X2+ 28.65 X3- 1.98 X1X2+ 13.72 X1X3- 40.11 X2X3- 261.82 X1X2X3+ 164.76 X1X2 (X1-X2)- 321.30 X1X3 (X1-X3)+ 96.22 X2X3 (X2-X3) (3.17)

Water Vapour Permeability = 0.97 X1+ 0.83 X2+ 0.97 X3+ 1.33 X1X2- 0.55 X1X3- 0.085 X2X3- 0.96 X1X2X3+ 5.37 X1X2 (X1-X2)- 1.48 X1X3 (X1-X3)+ 0.090 X2X3 (X2-X3) (3.18)

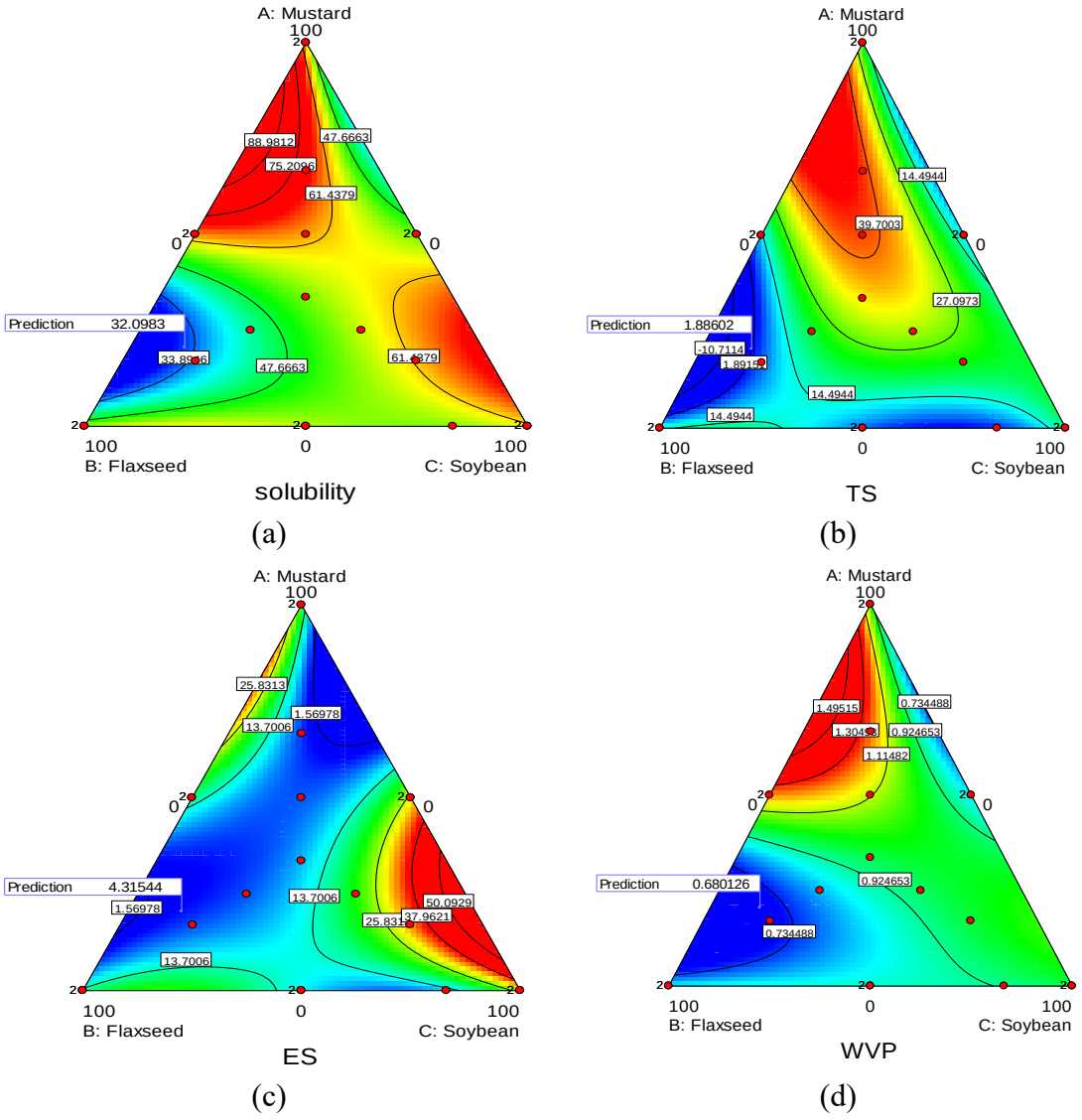


Fig. 3.3. Contour plot of (a) solubility (b) tensile strength (c) elongation at break and (d) water vapor permeability of biopolymeric films

3.5.3. Effect of operating parameters on properties of biopolymeric film

3.5.3.1. Water Solubility of biopolymeric films

Water solubility describes the amount of total soluble matter dissolves and remains after submersing the film into water for 24 h. This property depends completely on the amount of bound and unbound water present in the film. The Model F-value of 7.89 implies the model is significant with the Values of "Prob > F" less than 0.05. Table 3.6 showed the results variated in between the range of 29.28% to 69.10%. The solubility was seen to increase with increase in the percentage of soybean seed meal. Similar reasons were explained by Banker, (1966) states that higher the degree of dissolution of soybean

proteins, higher is the uncoiling of the soybean proteins. The lowest value was found in the control flaxseed seed meal with 0% mustard seed meals and 0% soybean seed meal whereas the solubility was found maximum in sample made with equal amount of mustard seed meal and flaxseed meal with 0% of soybean seed meal. The defatted mustard seed meal was found in range of 25.35±1.02% to 34.4±1.81%. The films treated were treated with High-pressure homogenization, Irradiation and Ultrasound treatment have seen to be no such effect on water solubility (Hendrix et al., 2012). The solubility index of the flaxseed-meal-based films are very high as recorded as 99.3 (Bangar et al., 2021).

3.5.3.2. Tensile strength of biopolymeric films

Tensile strength is the most important property that affects the shelf life of the food product covered inside the film. The mechanical properties are required to study whether the packaging biopolymeric film can withstand the stress of the external and barrier outside (Rao et al., 2010). The results of the tensile strength for 20 runs ranged from 3.09 to 44.34 kPa as presented in Table 3.6. This large variation in the readings is due behavioural features of different oilseed meals after combination. The mustard seed meals had more impact on the tensile strength than flaxseed or soybean seed meals. The mechanical strength of film was found highest with combination of 50% mustard seed meal along with 25% of flaxseed and 25% of soybean seed meal each. Whereas, the tensile strength was found lowest in the film with 50% combination of flaxseed and 50% soybean seed meals and 0% mustard seed meals. Similar trend with 1.3 to 5.5 MPa were found by Hendrix et al., (2012). The flaxseed meal protein film had the tensile strength values 13.12 MPa when added with 2 g fructose (w/w) (plasticizer), 0.03 g ferulic acid (crosslinker) and lemongrass as antimicrobial (w/w) (Lee et al., 2016).

3.5.3.3. Elongation of the biopolymeric film

Elongation at break is the study on ability of flexibility the packaging material without crack when shapes of material are changed. Elonagation is always studied parallel to the tensile strength. Elongation at break is the ratio of change in the length of the biopolymeric film and the initial length before the rupture of the tested film. The results of elongation at break were Model F-value of 3.97 implies the model was found significant with values of "Prob > F" less than 0.0500 indicate model terms were significant. The elongation at break ranges from 1.87% to 35.52%. Similarly, 0.9 to 18.1% range of elongation was recorded byHendrix et al., (2012). The film run 12 had the highest value

of elongation whereas the film run 9 had the lowest elongation value as shown in Table 3.6. The Elongation values was found 61.90%, better when the Flaxseed meal protein film added with 2 g fructose (w/w) (plasticizer), 0.03 g ferulic acid (crosslinker) and lemongrass as antimicrobial (w/w) (Lee et al., 2016).

3.5.3.4. Water Vapour Permeability of biopolymeric films

Water vapour permeability is the property that explains the amount of mass transfer (water vapour) from high humidity to low humidity enabling the stability of film in the presence of high moisture bearing food products. This property also studies the moisture loss thus helps in prevention of mobility of volatiles from wrapped food products to films or vice versa (Zhang et al., 2015). Thickness plays a vital role in studying water vapour permeability and transparency of the film. Since protein are the main component of the oilseed meals based biopolymeric films, the films have hydrophilic property to retain moisture (Nandane and Jain, 2018). Thus, the water vapour permeability of the biopolymeric film decreases with increase in thickness of the film. The average thickness of film was found to have 0.59 mm.

The Model F-value of water vapour permeability was 5.04 implies that the model is found significant. The Values of "Prob > F" less than 0.05 indicate model terms as significant. The values of water vapour permeability of biopolymeric films ranged from 0.65×10^{-10} $gs^{-1}m^{-1}Pa^{-1}$ to 1.24×10^{-10} $gs^{-1}m^{-1}Pa^{-1}$ (Table 3.6). The water vapour permeability of flaxseed meal protein-based films had 2.09×10^{-9} g m/m^2 s Pa when added with 2 g fructose (w/w) (plasticizer), 0.03 g ferulic acid (crosslinker) and lemongrass as antimicrobial (w/w). And the water vapour permeability of the untreated defatted mustard meal films was 3.68±0.89 g mm/kPa h m^2 whereas treated with ultrasound was found 4.96±0.76g mm/kPa h m^2. The different treatments for polymer destruction of the defatted mustard seed meals had no as such observable effect seen on water vapour permeability (Hendrix et al., 2012).

Table 3.6. Responses of the dependent and independent variables for the biopolymeric films prepared with different concentrations of mustard, flaxseed and soybean meal

Runs	Proportion of the components in the ternary mixture						Dependent variables			
	In- real concentration (%)			In- Pseudo components			Responses			
	Mustard seed meal	Flaxseed seed meal	Soybean seed meal	X1	X2	X3	Y1	Y2	Y3	Y4
1	100.000	0.000	0.000	1.000	0.000	0.000	55.55	14.88	24.03	0.960
2	66.631	16.555	16.814	0.666	0.166	0.168	66.17	22.83	4.68	1.243
3	50.001	49.999	0.000	0.500	0.500	0.000	69.10	15.80	13.07	1.300
4	50.001	49.999	0.000	0.500	0.500	0.000	61.97	11.00	19.18	1.148
5	0.000	0.000	100.000	0.000	0.000	1.000	59.11	21.35	21.61	0.999
6	100.000	0.000	0.000	1.000	0.000	0.000	55.22	12.46	13.15	0.968
7	50.001	0.000	49.999	0.500	0.000	0.500	54.37	13.49	20.23	0.899
8	0.000	100.000	0.000	0.000	1.000	0.000	53.92	15.96	16.93	0.805
9	16.982	66.344	16.674	0.170	0.663	0.167	29.28	1.87	1.87	0.800
10	33.613	33.182	33.205	0.336	0.332	0.332	58.54	32.73	17.03	0.970
11	0.000	16.848	83.152	0.000	0.168	0.832	55.09	3.09	11.78	0.930
12	0.000	0.000	100.000	0.000	0.000	1.000	61.87	22.34	35.52	0.940
13	50.001	0.000	49.999	0.500	0.000	0.500	51.54	8.16	34.53	0.745
14	17.048	16.581	66.371	0.170	0.166	0.664	59.89	12.38	32.03	1.036
15	0.000	49.999	50.001	0.000	0.500	0.500	59.81	5.66	14.78	1.003
16	0.000	100.000	0.000	0.000	1.000	0.000	53.92	9.57	12.60	0.851
17	0.000	50.000	50.000	0.000	0.500	0.500	53.21	9.30	9.03	0.753
18	50.000	25.000	25.000	0.500	0.250	0.250	62.98	44.34	3.98	1.157
19	25.000	50.000	25.000	0.250	0.500	0.250	48.12	27.01	4.82	0.710
20	25.000	25.000	50.000	0.250	0.250	0.500	54.78	30.89	9.15	0.920

X1, X2 & X3 = Pseudo values of Mustard seed meal, Flaxseed meal and Soybean seed meal respectively. Y1 = Solubility (%), Y2 = Tensile strength (N/m), Y3 = Elongation at break (%), Y4 = Water Vapour Permeability$\times 10^{-10}$ ($gs^{-1}m^{-1}Pa^{-1}$)

Table 3.7. Regression coefficients of the response variables and analysis of variance of the cubic models[a]

Coefficients[b]	Response variables			
	Y1	**Y2**	**Y3**	**Y4**
Model	1101.76	2359.71	1332.69	0.41
Linear Mixture	91.04	299.18	448.62	0.065
Quadratic				
X1X2	154.17	6.90	0.62	0.15
X1X3	40	135.63	17.60	0.025
X2X3	1.19	105.46	131.25	6.08×10^{4}
Cubic				
X1X2X3	25.37	1128.39	84.13	1.944×10^{3}
X1X2(X1-X2)	532.46	415.09	68.99	0.086
X1X3(X1-X3)	96.06	20.39	407.14	7.404×10^{3}
X2X3(X2-X3)	9.76	50.09	59.67	5.327×10^{5}
R-Squared	0.8766	0.9298	0.7814	0.8194
Lack of Fit	100.05	81.25	70.17	0.033
Adjusted R^2	0.7655	0.8667	0.5846	0.6569
F ratio of the model	7.89	14.73	3.97	5.04
Prob>F	0.0017	0.0001	0.0213	0.0093
Press	5.618×10^{5}	2.954×10^{5}	13281.33	17.34

[a]$Y = \beta_1X1 + \beta_2X2 + \beta_3X3 + \beta_{12}X1X2 - \beta_{13}X1X3 - \beta_{23}X2X3 - \beta_{123}X1X2X3 + \gamma_1X1X2(X1-X2) - \gamma_2X1X3(X1-X3) + \gamma_3X2X3(X2-X3)$

[b]Coefficient of determination.

3.5.3.5. Colour analysis of the biopolymeric films

The data colour measurement in terms of L*, a*, b* and whiteness index (WI) of the biopolymeric films is shown in Table 3.8. The whiteness index helps in studying the whiteness of the film. Higher the scale, lighter color is the product. The L* gives the value in range of 0 to 100 as 0 for dark colored and 100 for light color of the sample. The value of (+) a and (-) a value gives red and green shade of the sample and (+) b and (-) b values provides yellow and blue shade in the sample respectively. Raw oilseed meals of flaxseed and mustard meals generally are dark in colour. This darkness was visually seen to affect the colour of the films after drying. Similar results found in the films developed by Crambe abyssinica/ gluten blended packaging film by Rasel et al., (2016).

The WI of the individual control biopolymeric films of Mustard (100:0:0), Flaxseed (0:100:0), Soybean (0:0:100) and of optimized film (20.50:67.16:12.33) were 36.02, 32.30, 39.62 and 32.58 respectively. The whiteness index was found highest in individual soybean meal film and lowest in individual flaxseed film. The L* values of the individual control biopolymeric films of Mustard (100:0:0), Flaxseed (0:100:0), Soybean

(0:0:100) and of optimized film were 42.79±2.67, 38.60±1.31, 47.93±3.50 and 38.93±0.28 respectively. The a* values of the individual control biopolymeric films of Mustard (100:0:0), Flaxseed (0:100:0), Soybean (0:0:100) and of optimized film were 2.54±1.36, 0.94±0.31, 11.33±1.55 and 1.17±0.06 respectively. The b* values of the individual control biopolymeric films of Mustard (100:0:0), Flaxseed (0:100:0), Soybean (0:0:100) and of optimized film were 3.85±3.02, 0.07±0.10, 13.54±4.83 and 0.22±0.11 respectively. The results showed significant effect of flaxseed meal on the optimized films. The WI, L*, a* and b* values of optimized film was observed to increase in comparison to the individual control flaxseed meal-based film since the optimized film suspension had higher amount of flaxseed percent in comparison to mustard and soybean seed meal percent. The soybean seed meals based biopolymeric films had highest value of WI, L*, a* and b* and was lighter in shade as compared to the optimized film.

The flaxseed meal protein-based film in combination with pectin and glycerol presented in paper Bangar et al., (2021) had L*, a* and b* value 59.47±0.15, 4.98±0.09 and 14.36±0.17 respectively whereas, L, a and b values of defatted mustard seed meal-based film in research paper of Hendrix et al., (2012) ranged from (69.9±2.7 to 77.6±0.37), (0.41±0.6 to 5.8±1.1) and (29.5±1.1 to 45.7±1.2) respectively.

Table 3.8. Color parameters (L*, a*, b*) and colour change of the biopolymeric films

Concentration Proportion of the components			**Colour Analysis**			
X1	**X2**	**X3**	**L***	**a***	**b***	**WI**
100	0	0	42.79±2.67	2.54±1.36	3.85±3.02	36.02
0	100	0	38.60±1.31	0.94±0.31	0.07±0.10	32.30
0	0	100	47.93±3.50	11.33±1.55	13.54±4.83	39.62
20.50	67.16	12.33	38.93±0.28	1.17±0.06	0.22±0.11	32.58

X1, X2 & X3 = Pseudo values of Mustard seed meal, Flaxseed meal and Soybean seed meal respectively.

3.5.3.6. Scanning electron microscopy (SEM)

The morphological properties of the biopolymeric films both individual oilseed meals as control and the film blended with all three meals was analysed through SEM optical parameters are shown in Figure 3.4. In the images of mustard seed meal based films (a & b), fine pores are visible on the surface of the films. The cross-section of the mustard are also seen uneven and highly porous. Similar results were found in Rasel et al., (2016).

The unevenly surface are also explained as pre-existing or formed during specimen cutting procedure. Images c and d are of flaxseed meal based films. There are cracks found on the surface of the films and the uneven globules can be the particles sticked on the surface after breakage of the films. The pores in the cross-sectional images were less porous than the mustard seed meals. Even in the literature of Lee et al., (2016), flaxseed meal protein film added with 1% lemongrass oil showed heterogeneous and compact structure on the surface and cross-sectional images. Images e and f are the imsages of soybean seed meal based films. The films had large cracks but cross-sectionally less porous in comparison to the mustard and flaxseed meal based films. Oh et al., (2016) found similar images in cold plasma treated defatted soybean meal based edible films. The treated films were rougher on surface but revealed decrease in sharpness on edges. Images g and h are the optimized film. The surface as well as the cross-sectional images obtained are found smooth and compact structure without cracks and the molecules of poteins are binded as well. The homogeneous suspension of the films always are good results on their structural integrity and subsequently on their desirable physical properties (Hendrix et al., 2012). The structural results were much better in comparison to optimized films and other individually formed films through oilseeds.

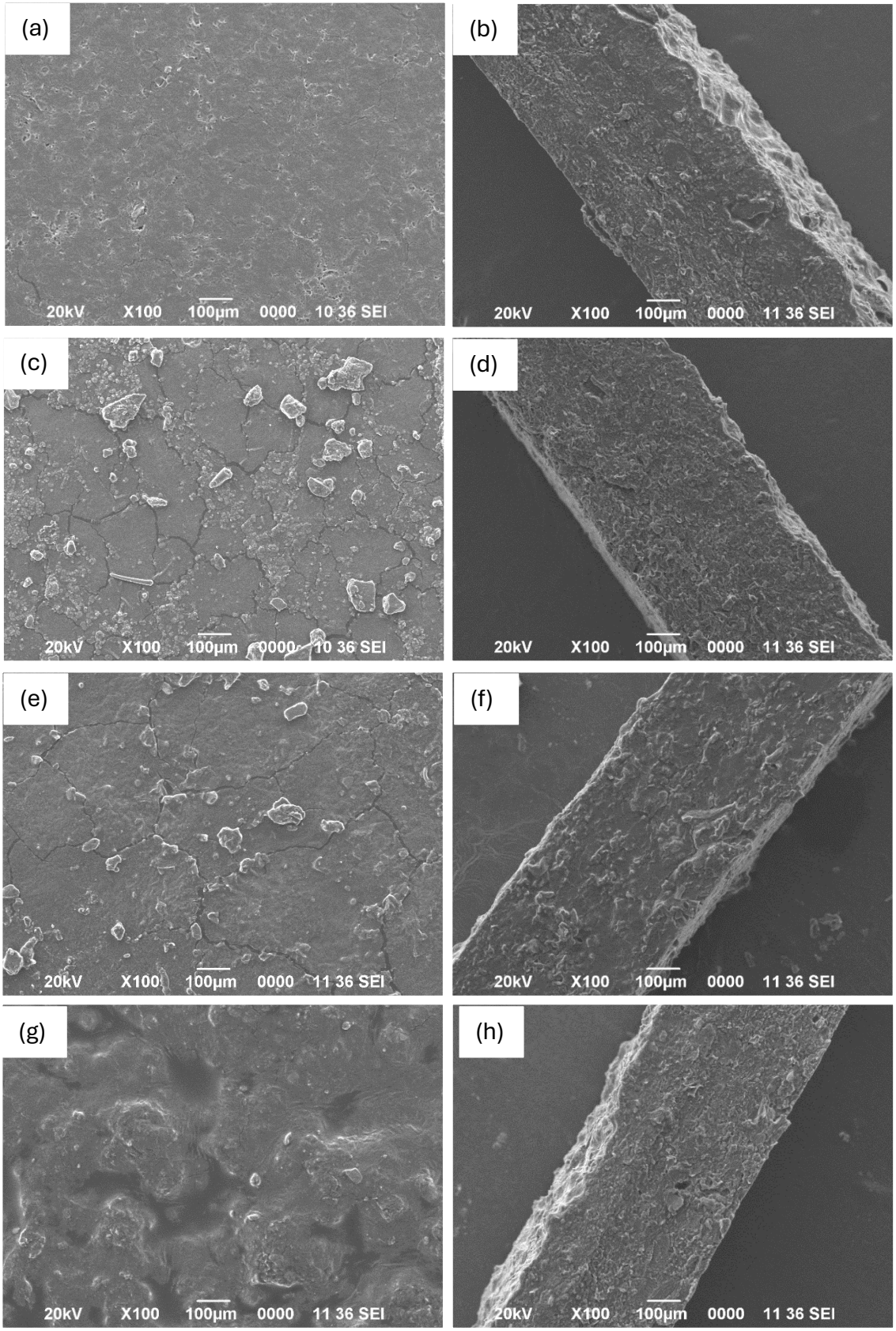


Fig. 3.4. Scanning Electron Micrographs at 100 × magnification of the surface and cross-section of mustard seed meal film (a, b), flaxseed meal film (c, d), soybean seed meal film (e, f) and optimized biopolymeric films (g, h) respectively.

3.5.3.7. Differential scanning calorimetry (DSC)

The Differential scanning calorimeter or DSC is helpful in measuring the heat flow rate variation in the sample with the reference sample (Dash et al., 2021). Films are subjected to different applications and temperatures while processing, thus study of the temperature limits through thermal attributes of the films is important (Lalnunthari et al., 2019). For the following samples of the biopolymeric films was subjected to heat up from 25˚C to 300˚C at 10 (K/min) difference.

DSC provides onset temperature (T_0), melting temperature (T_m), conclusion temperature (T_c) and enthalpy (ΔE) that are presented in Table 3.9. The comparative study of the individual mustard seed, flaxseed, soybean seed meal based films and optimized film are presented in Figure 3. Figure 4 illustrates the phase change for the mustard, flaxseed, soybean oilseed meals and optimized biopolymeric films where each one had two endothermic peaks of melting were detected. The first small peak seen can be associated as glass transition temperature (Tg) before onset temperature, which was recorded significantly similar in all graphs of DSC at temperature ranging between 25.122˚C to 27.548˚C as shown in Figure 3.5 and 3.6. The first endothermic peak (1), the onset temperatures of different films ranged from 25.6 ˚C to 50.0˚C can be attributed as dehydration temperature and the end temperature ranged from 120.9˚C to 144.5˚C. The melting temperature of all the biopolymeric films recorded ranged from 86.8˚C to 92.3˚C and the enthalpy ranged from -304.9 J/g to -337.2 J/g. As per observation, the individually developed flaxseed meal-based film had the highest melting peak with 92.3˚C followed by optimized films with 90.5˚C since the optimized films contains higher proportion of flaxseed meals in the combination of film forming suspension in comparison to other meals. The individually developed films from soybean seed meals had the least transition temperatures as well as enthalpy. For the second endothermic peak (2), the onset temperatures of different films were recorded from 199.1˚C to 202.1˚C and the end temperature ranged from 268.1˚C to 276.3˚C. The melting temperature of all the biopolymeric films recorded ranged from 238.0˚C to 241.9˚C and the enthalpy ranged from -153.9 J/g to -291.1 J/g. The optimized film transition temperature and enthalpy was observed to increase in comparison to flaxseed meal film but less than soybean seed meals. The melting point values were found significant decrease from 168°C to 162°C and from 72°C to 62°C when mustard seed meal powder was added to PLA and PEO polymers indicated the interference of mustard seed meal powder into chain formation of crystalline

phases of the polymers (Dai and Lim, 2015). The thermal analysis of the flaxseed meal films along with glycerol and pectin showed 62.10 ± 0.10°C (Bangar et al., 2021). Mikus et al., (2021) did the thermal analysis of Composite Films Based on Soy Protein Isolate and Oilseed Flours which exhibited that the film prepared with Soy protein without other oilseed flours addition showed the highest temperature stability with (99.98˚C), whereas films prepared with flaxseed flour reviled the lowest temperature stability at (53.20˚C). The presence of second peaks or multiple melting peaks are found commonly in the polymer-based films known as polymer decomposition or degradation or depolymerization of the films (Feng et al., 1998). The polymer leads to decomposition happens in the exothermic peak at higher temperature due to evaluation of CO, CH_2 and H_2O from polysaccharide films (Nair et al., 2020). Since, there was absence of exothermic peaks in the DSC graphs (Fig. 3.5 & 3.6), there was no polymer degradation observed in the oilseed meal based polymeric films.

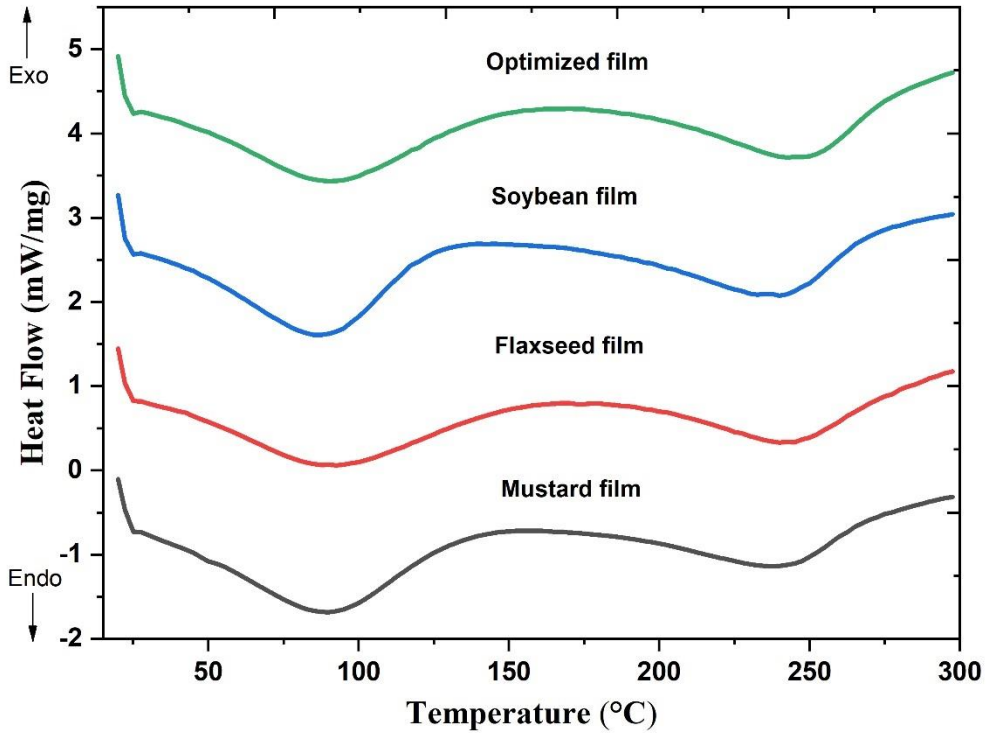
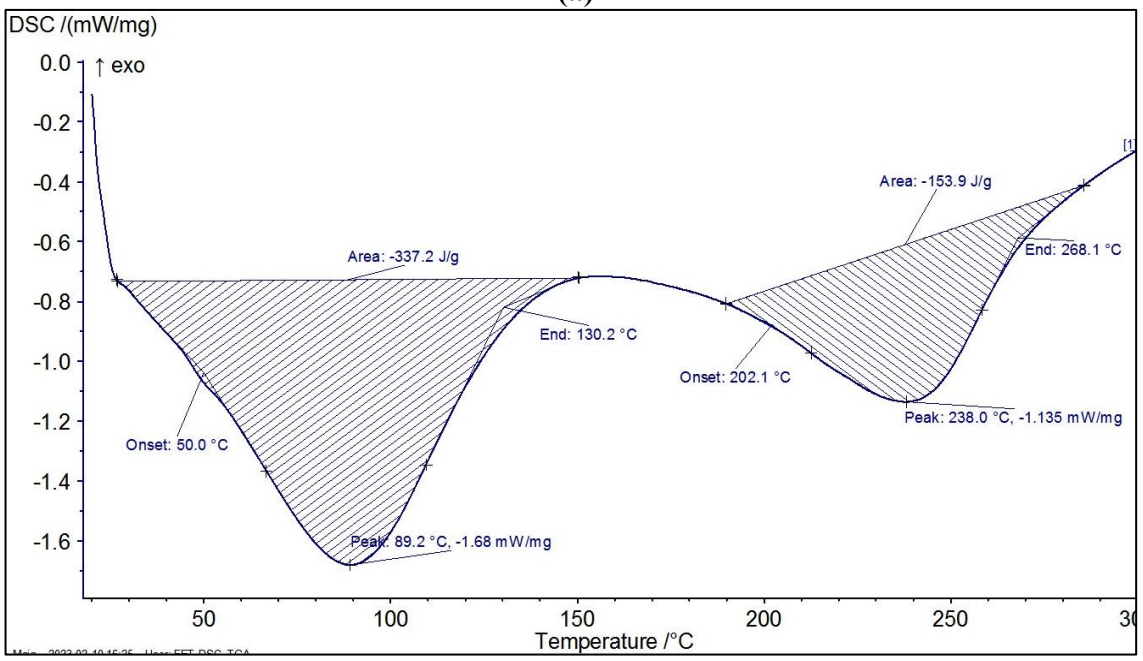
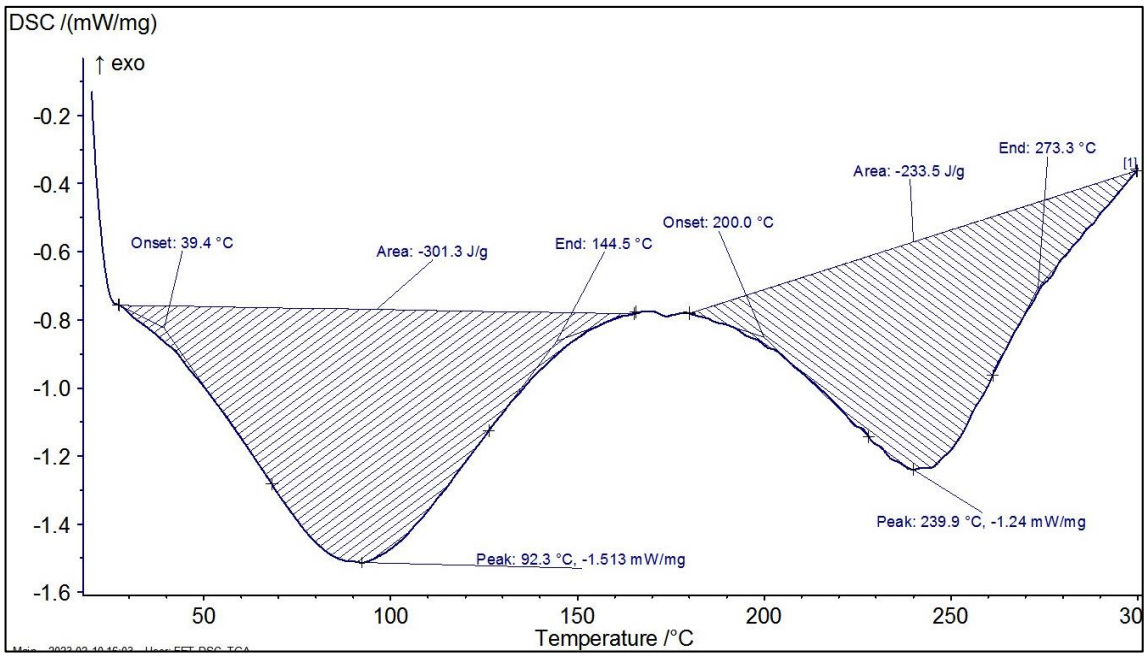


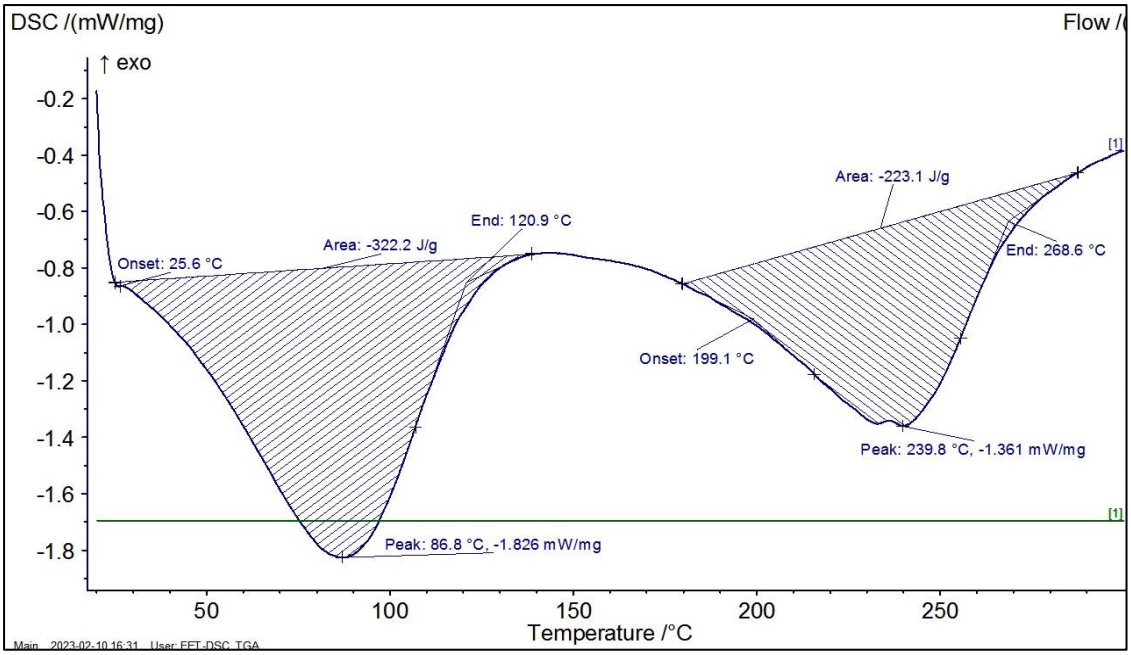
Fig. 3.5. Differential scanning calorimetry (DSC) curves for optimized, soybean seed meals, flaxseed meals and mustard seed meals based biopolymeric films.

(a)

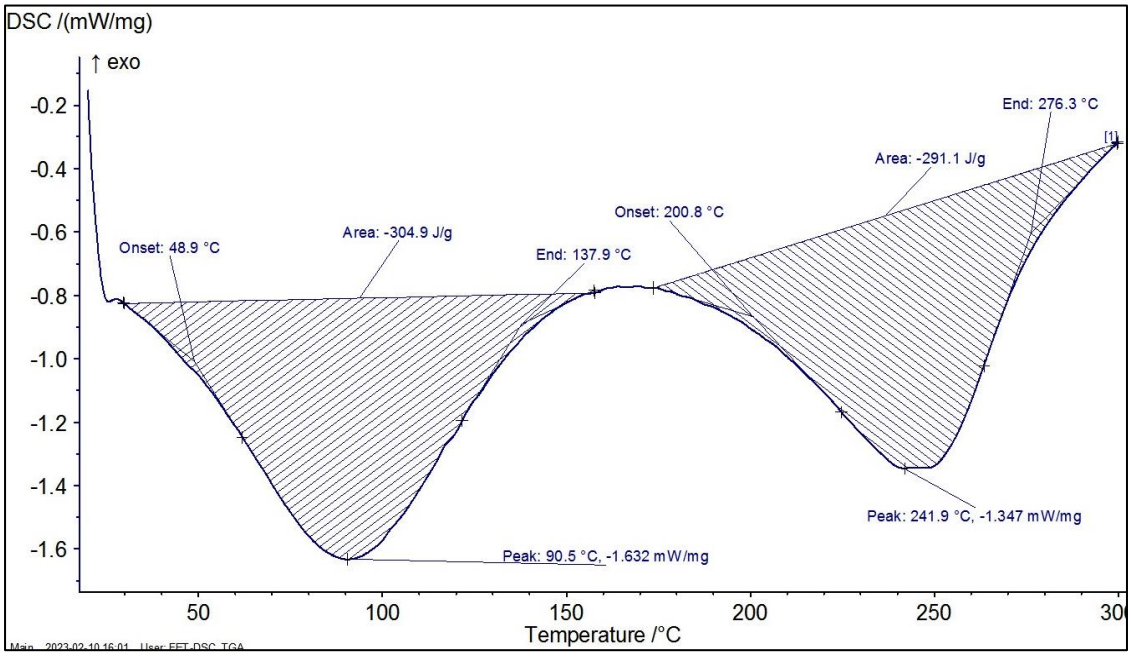

DSC /(mW/mg)
↑ exo
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
-1.6
Area: -337.2 J/g
End: 130.2 °C
Onset: 50.0 °C
Peak: 89.2 °C, -1.68 mW/mg
Area: -153.9 J/g
End: 268.1 °C
Onset: 202.1 °C
Peak: 238.0 °C, -1.135 mW/mg
50
100
150
200
250
Temperature /°C


DSC /(mW/mg)
↑ exo
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
-1.6
Onset: 39.4 °C
Area: -301.3 J/g
End: 144.5 °C
Peak: 92.3 °C, -1.513 mW/mg
Onset: 200.0 °C
Area: -233.5 J/g
End: 273.3 °C
Peak: 239.9 °C, -1.24 mW/mg
50
100
150
200
250
Temperature /°C

(b)



(c)



(d)

Fig. 3.6. Differential scanning calorimetry (DSC) curve for mustard seed meals (a), flaxseed meals (b), soybean seed meals (c) and optimized biopolymeric films (d) respectively.

Table 3.9. Thermal properties of the biopolymeric films

Film combinations			Transition Temperature (˚C)						Differential heat rate (mW/mg)		Enthalpy (J/g)	
			Peak 1			Peak 2			ΔH		ΔE	
X1	X2	X3	T_0	T_m	T_c	T_0	T_m	T_c	Peak 1	Peak 2	Peak 1	Peak 2
100	0	0	50.0	89.2	130.2	202.1	238.0	268.1	-1.68	-1.135	-337.2	-153.9
0	100	0	39.4	92.3	144.5	200.0	239.9	273.3	-1.51	-1.24	-301.3	-233.5
0	0	100	25.6	86.8	120.9	199.1	239.8	268.6	-182	-1.361	-322.2	-223.1
20.50	67.16	12.33	48.9	90.5	137.9	200.8	241.9	276.3	-1.63	-1.347	-304.9	-291.1

X1, X2 & X3 = Pseudo values of Mustard seed meal, Flaxseed meal and Soybean seed meal respectively.

3.5.3.8. Fourier-transform infrared spectroscopy (FTIR) analysis

For this research, to determine the molecular interactions between all the oilseed meals in the optimized biopolymeric films and comparative study in between mustard seed, flaxseed and soybean seed meals-based films, FTIR analysis was carried out in the range of 400 cm^{-1} to 4000 cm^{-1}. The graphs of FTIR are presented in Figure 3.7. The characteristic spectrum obtained for mustard seed meal-based films were in range 1087 cm^{-1} attributed to stretching bands of C-O stretching and 1084 cm^{-1} attributed for C-O linkage in C1 and C2. The flaxseed meal-based films few bands achieved of 832 cm^{-1} of C=Cl bending of Halo compound, 1179 cm^{-1} for C-O stretching in C2 and 1575 cm^{-1} for C=C stretching of cyclic alkene. The peaks found for soybean seed meal-based films were 1359 cm^{-1} for O-H bending, 1797 cm^{-1} for strong C=O stretching conjugated acid halide, 2904 cm^{-1} for C-H stretching of alkane. The peaks found for optimized biopolymeric film obtained in combination of all the three meals were found common like C=O stretching peak on 1797 cm^{-1} 2998 cm^{-1} for C-H stretching of alkane. The peaks were justified and found in similar range in the study reported in the literatures of Dai and Lim, (2015); Mikus et al., (2021); Guerrero et al., (2010); Tee et al., (2017) and Lan et al., (2020).

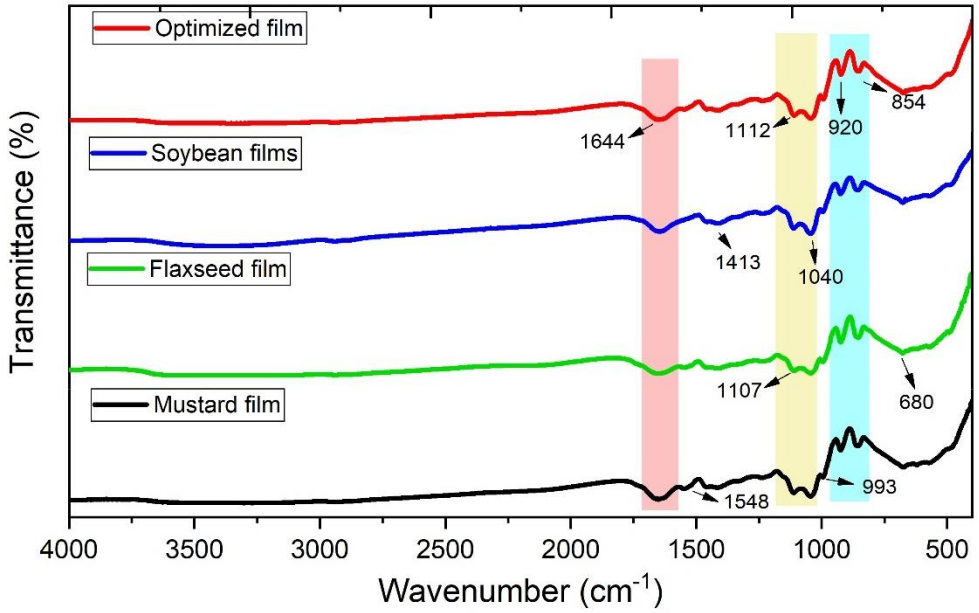


Fig. 3.7. Fourier-transform infrared spectroscopy graph for different oilseed meals and optimized biopolymeric films

3.5.3.9. Optimization and justification of the optimized film formation

The optimal biopolymeric film formulation was accomplished by mustard seed, flaxeed and soybean seed meals for studying their effect on physical, mechanical and properties. Mixture design was used for the optimization of the biopolymeric films. Based on the effect of independent variables (oilseed meals) on the response values for various properties of film, the optimal conditions obtained for formulation of biopolymeric film were acheived to be Mustard seed meal (20.50%), Flaxseed meal (67.16%) and Soybean seed meal (12.33%). This formulation was tested in triplicate experiments to validate these predicted conditions, and the results obtained were the actual values for properties were found similar to the predicted values as shown in (Table 3.10). These results shows that this formulation can be applied to prepare a successful biopolymeric film with good physical, mechanical and barrier properties for further practical utilisation.

Table 3.10. Predicted and experimental solutions for the optimized biopolymeric film suspension

X1	X2	X3	Y1	Y2	Y3	Y4	
20.50	67.16	12.33	32.09	1.88	4.31	0.68×10^{-10}	Predicted
			34.81	3.27	8.44	0.74×10^{-10}	Experimental

X1= mustard seed meal, X2= flaxseed meal, X3= soybean seed meal

Y1 = Solubility (%), Y2 = Tensile strength (N/m), Y3 = elongation at break (%), Y4 = Water Vapour Permeability$\times10^{-10}$ ($gs^{-1}m^{-1}Pa^{-1}$)

3.6. Conclusions

The functional properties of mustard seed, flaxseed and soybean seed cakes and their defatted oilseed meals were evaluated. The protein content for all oilseeds cake was quite high and increased after defatting for incorporation into food production as protein denaturation enhances the emulsifying capabilities. Water and oil absorption capacity was more in defatted oilseed meal compared to oilseed cake of mustard and soybean seed meal. This is a very desirable factor in bakery and meat products formation. The foaming capacity was increased after defatting of mustard seed and flaxseed cake. However, it decreased in case of soybean seed cake after defatting. Emulsion capacity was found highest in defatted soybean meal and emulsion stability was highest in flaxseed meal. A good emulsion capacity and stability in flours are quite helpful in preparation of whiteners for coffee, frozen desserts, and bakery foods. The least gelation concentration attained good results in all oilseed cakes and meals. A good binding tendency comes with higher least gelation concentration with the ability to bind protein, fat or starches enhancing good elasticity and plasticity for film formation and good viscosity for food product giving smooth texture and rheology to end product edible and non-edible bioplastics. The findings of this study will be helpful for deciding the suitability of oilseed cake and defatted meal for their direct utilization in new food product development as well as bioplastics formulation. In this study, mustard, soybean and flaxseed meal blend formulation was optimized to develop a biopolymeric film. The developed optimized film was characterized on the basis of its properties. The mixture design generated cubic model was presented for all responses and as further experimentally validated indicating that oilseed meal based blended biopolymeric film significantly affected different properties of films. The results of mechanical, permeability, morphological, thermal, and functional properties probably improved. After blending, the oilseed meals-based films in comparison to the individually formed films was formally viewed through Scanning

electron microscopy. The morphological images of optimized film were found more compact structure and surface of the film had homogeneous structure without cracks. The enthalpy recorded during thermal analysis of the optimized film was found to increase in comparison to other combination films. Several unique functional groups were found in FTIR analysis. The biopolymeric film with Mustard seed meal (20.50%), Flaxseed meal (67.15%) and Soybean seed meal (12.33%) along with addition of 2% Soy Lecithin as emulsifier and 75% Glycerol as plasticizer was compactable to produce a successful film. This research was successful applied mixture design and demonstrated well for the investigation, optimization, and fabrication of an industry-suitable, waste managed, oilseed based biopolymeric film blend. The film properties and shelf life can be further improved by addition of different additives, crosslinkers and antimicrobial components.