Chapter 4

Study the influence of natural gums and crosslinkers on the properties of oilseed meal based biopolymeric films

4.1. Introduction

One of the main industrial crops grown worldwide for the manufacture of oil is oilseed. The oilseeds produce a substantial amount of de-oiled meals as by-products. Proteins are abundant in defatted meals, therefore using them to create biopolymeric film material is an appealing substitute. The oilseed meals such as neem seed, pumpkin seed, hempseed, evening primerose, sunflower, crambe seed, carinata seed, flaxseed, soybean seed and sesame seed have been seen successfully used for developing packaging materials (Aramwit et al., 2022; Lalnunthari et al., 2019; Lee et al., 2016; Mikus et al., 2021; Lee et al., 2011; Newson et al., 2013).

Effective formation of film using only oilseed meal is a challenging task. The oilseed meals or isolate developed plastics/films are found brittle without plasticizers and emulsifiers (Guerrero et al., 2010; Mali et al., 2006; Newson et al., 2013). During development of oilseed meals based biodegradable films, the oilseed meals particles in the suspension tend to settle down eventually which affect film formation. The possible solution is to incorporate emulsifiers which can improve the textural properties as well as emulsion stability of oilseed particles.

Gums and resins are found abundantly in nature, and are extracted by wide variety of nature like microorganisms, plants, and animals, as well as from materials prepared by modification or blending of aforesaid natural structures and from synthetic polymeric materials with various crosslinkers (Dastidar and Netravali, 2012; Gandini and Belgacem, 2008; Hendrix et al., 2012; Mangaraj et al., 2019). As natural gums, crosslinkers also bid new openings in designing of efficient biopolymeric film based biodegradable and biocompatible polymeric materials with desirable properties such as strong bonding of different polymers (Dastidar and Netravali, 2012; Khattab and Arntfield, 2009).

Arabic gum, sometimes called gum acacia, comes from plants that are a derivative of the Acasia Seyal tree's exudate. With a complicated branch-on-branch structure made up of L-arabinose, D-galactose, L-rhamnose, D-glucuronic acid, and a minor amount of nitrogen-containing compounds, this non-toxic polysaccharide has a tetra-heteroglycan monomeric structure (Jani et al., 2009; Nair et al., 2020). Gum Arabic are found highly interactive with proteins (highly charged in nature) to form complexes due to their low linear charge density. The gum acacia is acidic in nature and behaves negatively charged in presence of wide pH range. Electrostatic interaction of between gum acacia and different

proteins such as canola protein isolate, soy protein isolate, and gelatin have been studied (Naderi et al., 2020).

Xanthan gum microbially originated extracellular hetero polysaccharide by the culture of microorganism *Xanthamonas campestris.* Xanthan gum's main structural component is a linear β−1,4 linked D-glucose chain that is replaced every other glucose residue with a charged trisaccharide side chain that has a glucuronic acid residue in between two mannose residues. The helix's outermost point, C(6), is where the inner mannose residue is often acetylated to stop aggregation (Veiga-Santos et al., 2005). Xanthan gums are specified as non-ionic gums with chemically short branched tri-heteroglycans in structure. Maillard-type reactions, also known as glycation, are carried out by hydrocolloids conjugating proteins between the reducing end carbonyl group in polysaccharides and the ε-amino groups in proteins (Chen et al., 1997). Literatures have effectively showed synergism interactions when combined with other natural products such as starch, gums, CMC etc. to produce a successful packaging. Gum content at its ideal level might enhance mechanical and barrier qualities. A combination of xanthan gum and acacia gum can produce film with advantageous synergy and promising film characteristics (Jain et al., 2015; Veiga-Santos et al., 2005).

Further, crosslinking of the biopolymers such as proteins and its interaction with other polymers has been observed an approach towards improving the mechanical and barrier properties of film (Sharma et al., 2018). Industrial citric acid as called as green crosslinkers, is a natural crosslinkers procured from an aerobic organism *Aspergillus niger* and extracted from citrus fruits through different fermentation processes (Salihu et al., 2021). Conversely, glutaraldehyde is an artificial crosslinker made in an industrial setting by hydrogen peroxide-catalyzed cyclopentene oxidation, which is accomplished in the presence of heteropoly acid catalysts based on tungstic acid (Chandler et al., 2021; Hiroshi et al., 1999). Citric acid and glutaraldehyde are both water soluble and economically cheap (Migneault et al., 2004; Reddy and Yang, 2010).

Citric acid structurally tricarboxylic acid and one hydroxyl group compound, when added with isolated proteins in any solution as crosslinkers, it acts on lysine. During the crosslinking reaction, the hydroxyl groups of polysaccharide and the two carboxyl groups of citric acid gets crosslinked by covalent intermolecular di-easter linkages (Ma et al., 2018). Modification in the proteins leads to increase the reaction sites more available for

crosslinking with citric acid before any thermal processing for drying (Newson et al., 2023). Structurally, glutaraldehyde is a linear, 5-carbon dialdehyde. Glutaraldehyde added with proteins in basic or neutral medium, its α, β-unsaturated molecules predominate on protein molecules. The main amines and aldehyde molecules combine to generate imines, or Schiff bases, which are stabilised by resonance. When the concentration of proteins is greater than that of the cross-linking agent, amine attaches itself to the ethylene bond of α, β-unsaturated polymers, resulting in a secondary Michael reaction (Marquie, 2001).

Therefore, present work was planned to promote a novel method for preparing an oilseed meal based biopolymeric film using natural gums (acacia & xanthan gum) and crosslinkers (citric acid and glutaraldehyde). The aim of the study was to produce the best combination of natural gums and the best crosslinkers suited for the development of the oilseed meal based biopolymeric film. The study was also conducted to understand the benefits of interactions between the oilseed meals with gums and crosslinkers on the film development through studying the physico-chemical characteristics of the films. The relationship between the structural modifications of the films after addition of natural gum and crosslinkers was also examined.

4.2. Materials and Methods

4.2.1. Materials

Food grade acacia gum (AG) and xanthan gum (XG) was obtained from Hi Media Laboratories, India; whereas, citric acid (CA) and glutaraldehyde (GL) were obtained from Merck, India. The viscosity of gums was analyzed using Digital Viscometer (DV-79) and viscosity was observed for 1% Acacia gum= 3.10 cps and 1% Xanthan gum in 1% KCl= 3900 cps. We purchased meals made from mustard, flaxseed, and soybean seeds at the Tezpur, Assam, India, local market. The film development employed n-hexane, glycerol and soy lecithin which were purchased from Merck Chemicals, India. For this investigation, laboratory reagent compounds were all used.

4.2.2. Methods

4.2.2.1. Raw material collection and formation

The sample of flaxseed, mustard and soybean seed cakes were procured from nearby market Tezpur, Assam, India. The collected samples were dried at 60 ˚C in tray drier (IKON, Delhi, India) and cleaned manually to remove unwanted impurities. The raw

oilseed cakes were grinded into oilseed powdered meals and sieved via 45 μm mesh sieve, packed and stored in airtight containers at 4˚C.

4.2.2.2. Defatting of the oilseed meal sample

To reduce the oil content to 1%, each dried oilseed meal was individually defatted over a Soxhlet apparatus using n-hexane at a ratio of n-hexane: flour (30:1, v/w). The finished defatted meals were kept in airtight containers at 4˚C for further analysis after being dried at 40˚C in a tray drier (IKON, Delhi, India) for a whole night to remove any remaining n-hexane from the meal.

4.2.3. Development of oilseed meals and gums (Acacia gum and Xanthan gum) based films

In the present study, the oilseed meals-natural gums based blended biodegradable films were prepared by using the solvent casting method. The combination of oilseed meals used in the film formulation were selected from previous chapter as mustard seed meal (20.50 w/w%), flaxseed meal (67.15 w/w%) and soybean seed meal (12.33 w/w%). The methodology for oilseed meal/natural gums was chosen based on previous results to produce and characterize the biopolymeric films. For the development of oilseed meals-gums films, the natural gums (Acacia & Xanthan powder) were priorly mixed and gelatinized in 50 ml distilled water along with Glycerol (75% w/w) as plasticiser at 80˚C on magnetic stirrer (900 rpm) (ABDOS MS H280 Pro, India) for 15 min and on the other hand, the known quantity of the oilseed meals is mixed separately in the rest 50 ml distilled water on magnetic stirrer (900 rpm) at 50˚C for 10 min. At last, the natural gums-glycerol suspension is gradually added into the oilseeds meal suspension through constantly stirring at 900 rpm for uniform mixing for another 15 min at 50˚C. The entire mixture is heated to 90˚C for 30 minutes, stirring occasionally, in a hot water bath (Modern, New Delhi), and then chilled in ice cold water. After adding 2% w/w soy lecithin to the suspension as an emulsifier, the mixture is agitated for 10 minutes at 50˚C before being cast in petri plates. The air bubbles were eliminated before casting. With the help of a portable spirit level, the film petri dishes (150×25 mm) was casted and dried at 65˚C in hot air oven (IGene Labserve IG-95HA0) for 3 days to produce an even and smooth film. After drying, the film is peeled off carefully from petri plates and stored in zipped pouches into the desiccator with dried silica gel for further analysis.

4.2.3.1. Experimental design of oilseed meals-gums (AG: XG) based film

One of the most popular and commonly used experimental designs is the complete factorial design. Studying the combined impact of the factors (or process/design characteristics) on a response is made possible by factororial designs. All feasible level combinations for every component are included in a complete factorial design experiment (Antony, 2014). Full factorial design (FFD) investigates the effect of all the factors and their interactions on the results. The design comprises of level termed as high (+1) and low (-1) (Das et al., 2018).

To achieve an optimized oilseed meal-natural gum based blended films, a 4^2 (two factor and 4 level) factorial design was applied with 16 experimental runs with 0 replications. Full Factorial Design for the development of film is presented in Table 4.1. The amount of gum acacia (G1, %) and gum xanthan (G2, %) were selected as independent variables (Table 4.1). Whereas, the responses such as solubility (Y1), tensile strength (Y2), elongation at break (Y3) and water vapour permeability (Y4) were selected as the dependent variables (Table 4.2) was helpful in studying the development of the best compactable oilseed-natural gums blended biopolymeric films. Design Expert® software (2018), Stat-Ease Inc., USA) was used for statistical estimation of experimental design.

The primary effect of both factors and their interaction on answers was found for a 4^2 (two factor and 4 level) factorial design. The optimized film obtained after analysis were further added with crosslinkers to study their coadjuvant effect on the optimized films.

Table 4.1. Concentrations of acacia gum and xanthan gum with oilseed meals used in the film formulation determined according to full factorial design.

Oilseed meals	Concentration (%)	
	Mixture components	
	AG (%)	XG (%)
	1.5	1
	0	0
	1	0.5
	0.5	1
	0.5	1.5
Mustard seed meal	0.5	0.5
(20.50%),	0.5	0
Flaxseed meal	1	1.5
(67.15%),	0	1.5
Soybean seed meal	1	0
(12.33%)	0	0.5
	1.5	1.5
	0	1
	1.5	0.5
	1	1
	1.5	0

Amount of oilseed meals (w/w) added to 100 mL distilled water.
The value represents the concentration distribution of the gums with respect to total amount of oilseed meals in suspension.

4.2.4. Development of oilseed meals-gums based film added with citric acid and glutaraldehyde as crosslinkers

The optimized suspension obtained after oilseed meal-natural gum film analysis was further used in film development in addition with Citric acid and Glutaraldehyde crosslinkers as 2, 4, 6, 8 & 10% w/w of the sample. The process of film development was to prepare a crosslinked film at different ratios coded as shown in Table 4.2.

Previously, the developed films of oilseed meal-gums were added with two different crosslinkers in different proportion in the film suspension as shown in Table 4.1.

Solvent casting was used to create blended biodegradable films based on oilseed meals-natural gums. For the addition of the natural gums (Acacia & Xanthan powder) and crosslinkers (Citric acid and Glutaraldehyde), they were taken in appropriate quantity and were priorly dissolved and in 50 ml distilled water and heated at 80˚C for gelatinization on magnetic stirrer (900 rpm) (ABDOS MS H280 Pro, India) for 15 min and on the other part, the known quantity of the oilseed meals along with Glycerol (75% w/w) as plasticiser is mixed separately in the rest 50 ml distilled water on magnetic stirrer (900 rpm) at 50˚C for 10 min. Afterwards, the natural gums crosslinkers suspension is gradually added into the oilseeds meal suspension through constantly stirring at 900 rpm for uniform mixing for another 15 min at 50˚C. The entire mixture is heated to 90˚C for 30 minutes, stirring occasionally, in a hot water bath (Modern, New Delhi), and then chilled in ice cold water. Finally, the suspension is mixed with 2% w/w soy lecithin, an emulsifier, and poured into petri plates after being agitated for 10 minutes at 50˚C. Before casting, the air bubbles were removed by allowing the suspension to remain undisturbed for ten minutes. To create uniform and smooth films, 150 x 25 mm film petri dishes were dried in a hot air oven at 70˚C for three days with the aid of a portable spirit level. Following drying, the film is carefully removed from the petri plates and placed in zip pouches containing dried silica gel inside the desiccator for additional examination (Mohsin et al., 2020).

Ten different films were developed in addition with two types of crosslinkers separately and were further analysed based on the responses such as solubility (Y1), tensile strength (Y2), elongation at break (Y3) and water vapour permeability (Y4) were selected as the dependent variables to study the comparison between different types and between different ratios of crosslinkers addition.

Table 4.2. Formulation of oilseed meals-gums crosslinkers based biopolymeric films

S.No.	Oilseed meals[a]	Natural gums[b]	Citric acid added films[c]	Glutaraldehyde added films[d]
1	Mustard seed meal (20.50%), Flaxseed meal (67.15%), Soybean seed meal (12.33%)	Acacia Gum: Xanthan Gum (0.5:1.5% w/w of oilseed meal)	CAF2%	GLF2%
2			CAF4%	GLF4%
3			CAF6%	GLF6%
4			CAF8%	GLF8%
5			CAF10%	GLF10%

[a] Oilseed meals of mustard, flaxseed and soybean ratio fixed as in pre-determined study for all the films.
[b] Gum Acacia and Gum Xanthan ratio 0.5:1.5% (w/w) of the oilseed meal sample is constant for all the films.
[c] The CAF (2-10%) represents the percentage addition of the Citric acid with respect to the total amount of oilseed meals in 100 mL of film suspension.
[d] The GLF (2-10%) represents the percentage addition of the Glutaraldehyde with respect to the total amount of oilseed meals in 100 mL of film suspension.

4.2.5. Characterization of biopolymeric films

The film developed with oilseed meals added with gums and crosslinkers were characterized with following properties.

4.2.5.1. Film thickness, moisture content and solubility

The moisture content of the biopolymeric film was determined by calculating the difference between the sample's initial and final weight loss following a 24-hour drying process at 105°C (Horowitz, 2005). The thickness readings were taken at six random locations on films from micrometre (Alton M820-25, China) with a sensitivity of 0.01 mm (Pelissari et al., 2012).

The film samples, measuring 2 by 2 centimetres, were dried at 105°C and then immersed in 40 ml of distilled water, maintaining room temperature throughout the day with frequent stirring. Subsequently, the damp samples were extracted from the water and subsequently dried in a hot air oven at 105°C for an additional 24 h. Calculations were made on the sample's solubility using equation 4.1 (Ojagh et al., 2010).

$$TSM\ (\%) = \frac{initial\ dry\ weight - final\ dry\ weight}{initial\ dry\ weight} \times 100 \qquad (4.1)$$

4.2.5.2 Water Vapor permeability

The glass beaker containing dried silica crystals at 100% relative humidity was sealed with the film specimen outside. The glass beaker was then put into the desiccator, which was filled with a 97% relative humidity saturated K_2SO_4 solution. The temperature of the desiccator was kept at room temperature. Through the change in weight of the glass beaker sealed with film samples, which was monitored every 24 hours for a week, the water vapour transfer rate (WVTR) was determined. The weight gain slope v/s time slope was calculated and divided by the film area (Wu et al., 2013). The water vapour permeability was calculated of using the equation 4.2.

$$WVP\ (g/Pa\ h\ m) = \frac{WVTR}{P(R1-R2)} \times X \quad (4.2)$$

Where, P represents the water's saturated vapour pressure (Pa) at room temperature.

The driving force under the experimental circumstances was considered to be [P (R1 – R2)] = 3073.93 Pa. R1 = relative humidity of the desiccator with K_2SO_4 solution. R2 = relative humidity of the cup with dried Silica crystals. X = thickness of film (m).

4.2.5.3. Mechanical properties

Based on the film's elongation and textural characteristics, its mechanical properties were computed. For the film examination, the texture analyzer (TA-HD Plus Stable microsystems, UK) was employed. The analyser was preconfigured to operate in tension mode with a weightlifting probe set to 5 kg and pre-test, test, and post-test speeds of 5 mm/s and 1 mm/s, weightlifting. Three separate runs or triplicate of the biopolymeric film (6 x 2 cm) analysis were conducted. The greatest power used to stretch the film and the separation between the grips prior to fallout were noted. The mechanical properties was calculated through equation (4.3) and (4.4) (Zavareze et al., 2014).

$$Tensile\ strength\ (MPa) = \frac{Maximum\ force\ of\ the\ film\ (N)}{Cross\ sectional\ area\ of\ the\ film\ (m2)} \quad (4.3)$$

$$Elongation\ at\ break\ (\%) = \frac{Distance\ of\ rupture\ of\ film}{Onset\ distance\ of\ the\ separation\ of\ film} \times 100 \quad (4.4)$$

4.2.5.4. Color and Scanning Electron Microscopy (SEM)

Utilising a Hunter Calorimeter (Ultra scan VIS; Hunter lab, USA), the biopolymeric films' colour was examined. White and black standard tiles were used to

calibrate the Hunters Calorimeter. The colour scale was established using L* (L*=0) as black and (L*=100) as white, and the acquired colour values were recorded as L*, a*, and b*. The chromaticity parameters were identified as "a* and b*" for "(–a) greenness; (+a) redness and (–b) blueness; +b) yellowness," respectively. The whiteness index (WI) was measured as presented in literature of Lee et al., (2016) using Eq. (4.5).

$$WI = 100- [(100-L)^2+ a^2+ b^2]^{0.5} \quad (4.5)$$

Scanning electron microscopy was used to examine the surface morphology of the biopolymeric film. In a hot air oven set at 60˚C for five hours, the films were dried before scanning. Then, before gold was deposited, the films were dried and finely sliced for cross-sectional and surface characterization. Using a JSM 6390LV scanning electron microscope (JEOL, Japan), the images from the biopolymeric films were scanned (Deepa et al., 2016).

4.2.5.5. Thermal properties

In order to investigate the thermal properties of the biopolymeric films, 0.01 g of the film sample was sealed in a pan and put into a Differential scanning calorimetry (DSC) machine chamber. The film sample was heated at a rate of 10.0 k/min from 20˚C to 300˚C using a Differential Scanning Calorimetry (214, Polyma, NETZSCH, Germany) (Lalnunthari et al., 2019).

The material of the crystalline phase was identified through X-ray diffraction of the film sample, and cell dimensions were measured. An X-ray diffractometer (BRUKER AXS D8 FOCUS) was used to analyse the biopolymeric film. An X-ray beam operating at 100 mA and 50 kV was directed at the dried film sample. The area of the crystalline and amorphous regions was determined using the 2 theta curve, which ranges from 10 to 80 degrees. The diffraction patterns were obtained using step scanning mode with 2θ = 5–60º, 5 s/step, and a step of 0.02º. The crystallinity degree was computed through the Eq.4.6 (Yumnam et al., 2023).

$$Degree\ of\ crystallinity = \frac{Area\ of\ crystalline\ peak}{Total\ area\ under\ curve\ (crystalline+amorphous)} \times 100 \quad (4.6)$$

4.2.5.6. Fourier-transform infrared spectroscopy (FTIR)

The biopolymeric films were subjected to infrared spectroscopy in order to determine the functional groups or chemicals compounds contained inside. The Bruker Equinox 55 spectrometer (Bruker Banner Lane, Conventry, Germany) was used to

evaluate the film pallets using Fourier transform infrared spectroscopy in the 400–4000 cm-1 range.

4.2.6. Statistical analysis of the developed biopolymeric films

To create oilseed meals-gums biopolymeric films, Software Design Expert version 7.0.0 (Statease Inc.) was utilised. Various mathematical models and three-dimensional graphs were employed to examine the impact of dependant variables. ANOVA was performed to compare the means of the data produced by significance level of p~0.05 after the polynomial model was employed for 16 runs in order to further investigate relativity.

Based on an analysis of the main and interaction effects, the data were fitted into a first-order linear regression model that included the main factors and their interaction terms. The experimental equation of the response variables was then calculated using the cubic model equation for the two components, ignoring the factors that had no effect on the response variables as shown in equation (4.7).

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_{12} AB + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{12} A^2 B + \beta_{21} AB^2 + \beta_{10} A^3 + \beta_{20} B^3 \quad (4.7)$$

The term Y represents different response variables. A and B represent the coded factors of gum acacia and gum xanthan, respectively, and βn denotes the parameters of the linear and crosslinked product of the model. Equations with binary coefficients βij show synergistic effects by having positive or negative values. β0, β12, β11, β22, β12, and β21 all exhibited the intercept effect.

In addition, the crosslinked biopolymeric film that was produced was examined using IBM SPSS Statistics 23 software and an ANOVA to compare the means of the obtained data with a significance level of p~0.05.

4.3. Results and Discussion

a) To study the effect of acacia and xanthan gums on the properties of the biopolymeric films

4.3.1. Model fitting for synthesis of oilseed meals-gums (AG: XG) blend films

In accordance with the complete factorial design, blended oilseed meal and natural gums were developed to create bubble-free, homogenous films with a smooth surface. Individual oilseed meals yielded biodegradable films, however these films proved to be fragile. Oilseed meals were blended to produce smooth films, however the surface of the

films had an oily, wet look that might shorten the films' shelf life at ambient temperature was observed in earlier chapters. The blended suspension of oilseed meals (mustard, flaxseed, and soybean seed meals) was enhanced by the addition of Arabic gum and Xanthan gum in varying ratios, together with soy lecithin and glycerol, to overcome these restrictions. Table 4.1 shows how each constraint (natural gums) was prepared for oilseed meal film suspension. The coded matrix of the natural gum's ratio for the addition in film suspension and its respective weight among the experimental values of water solubility, tensile strength, elongation at break and water vapour permeability are presented in Table 4.3. The cubic model's analysis of variance and regression coefficients for the response variables of the oilseed meals-gums (AG: XG) are displayed in Table 4.3.

In order to evaluate the precision of the fitted cubic model employed in the oilseed meals-gums film creation process, Table 4.3 presents several ANOVA components, including sum of square, coefficient of variance (R^2), p-value, adjusted R-squared, residual values, and so forth. Since there were no central point found in the full factorial design, the lack of fit was also not found in the ANOVA. To understand the variation of points in linear plot, an experimental verses regression model was plotted as shown in Fig. 4.1 (a-d). The presence of non-significant values depicts the least effect of the factor on the developed films.

In the Eq. 4.9, 4.10, 4.11, and 4.12, the Full Factorial coded values of the individual and interacting effects of Acacia gum and Xanthan gum when added in oilseed meal based biopolymeric films. ANOVA provides the elements needed to create the final equations for the various characteristics of the biopolymeric films. By fitting the responses on a complete factorial design, these equations show the cubic models that resulted. Response variables and the mathematical model are produced from the created 2D and 3D plots between dependent and independent variables in order to examine the interaction process between the variables.

Percent Solubility (Y1) = 41.01 -2.62A -1.34B +2.22AB -4.98A^2+ 0.55B^2 -3.21A^2B - 0.61AB^2 + 1.53A^3 -1.72B^3 (4.9)

Tensile Strength (Y2) = 1.02 -0.0054A + 0.0012B + 0.0002AB -0.0088A^2-0.0004B^2 - 0.0005A^2B + 0.0007AB^2 + 0.0083A^3 -0.0021B^3 (4.10)

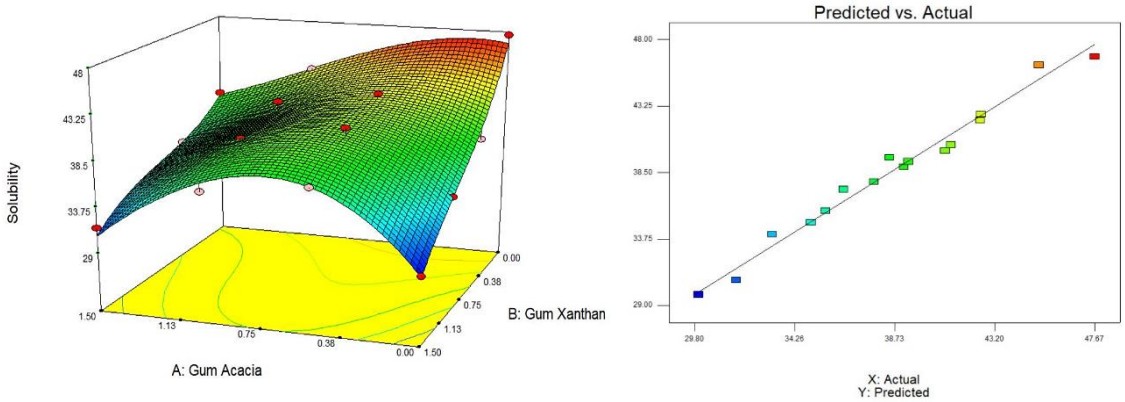
Percent Elongation (Y3) =13.65 +4.27A –9.77B +0.10AB –2.08A^2 +1.49B^2 +5.47A^2B +2.98AB^2 –7.23A^3 +10.14B^3 (4.11)

Water Vapour Permeability (Y4) =5.12+ 2.16A +2.76B -0.11AB -0.68A^2+ 0.02B^2 - 0.25A^2B + 0.64AB^2 -0.55A^3 -1.28B^3 (4.12)

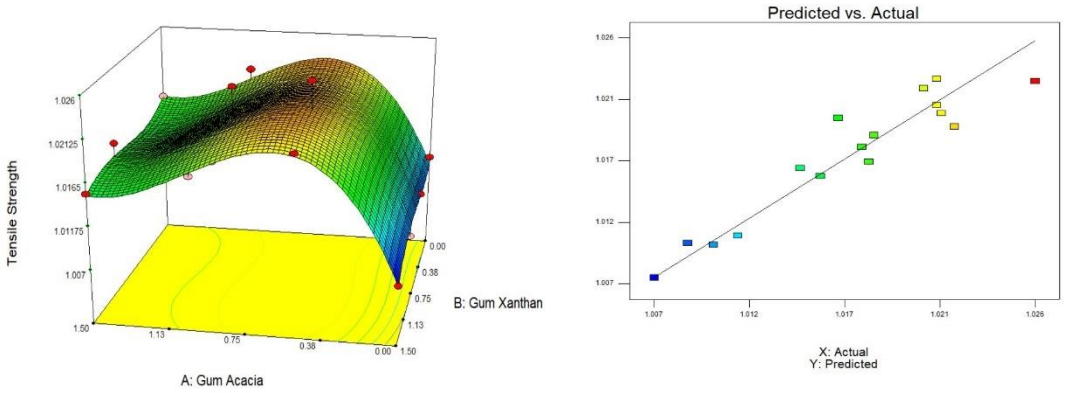
The response fitted on Model Eq. 4.9, 4.10, 4.11 and 4.12 show the solubility (Y1), tensile strength (Y2), elongation at break (Y3) and water vapor permeability (Y4) of the oilseed meals-gums biopolymeric films respectively, were found significant ($p<0.05$) by ANOVA as shown in Table 4.3. Further, the combined as well as individual gums effect are interpreted graphically in Fig. 4.1 (a-d) for all the responses. For the solubility, F-value, p-value and R^2 are 34.05, 0.0002 and 0.98 respectively. The Eq. (4.9) represents that A (gum acacia) and B (gum xanthan) shows negative influence on the solubility of the biopolymeric films whereas, the interactions term such as AB, B^2 & A^3 shows positive influence on the solubility. The results established by ANOVA shows the interaction terms (AB, A^2 & A^2B) also have significant effect on the biopolymeric film's solubility whereas, the influence of A and B on response (solubility) have non-significant effect.

The F-value, p-value and R^2 are noted for tensile Strength as 5.59, 0.0243 and 0.89 respectively. In the Eq. (4.10), A (gum acacia) shows negative influence whereas and B (gum xanthan) and interactions term such as (AB, AB^2, AB^2 & A^3) shows positive influence on the Tensile strength of the biopolymeric films. The interaction terms (A^2 & A^3) also have the significant effect on the biopolymeric film's tensile strength. The influence of A and B on response (tensile strength) showed are non-significant. The value of F-value, p-value and R^2 for elongation at break was recorded as 5.85, 0.0218 and 0.89 respectively. The Eq. (4.11) represents that A (Gum Acacia) along with interactions terms (AB, A^2B, AB^2, B^2 & B^3) shows positive influence whereas, B (Gum Xanthan) along with interactions terms A^2 and A^3 shows negative influence on the Percent Elongation of the biopolymeric films. The interaction terms (B^2 & A^2B) have significant effect whereas, the influence of A and B on response (elongation at break) showed non-significant effect on the biopolymeric film's elongation at break.

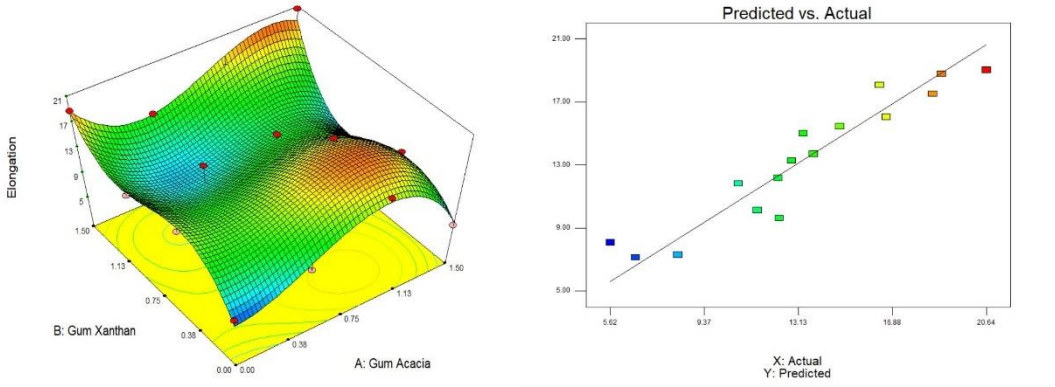
The Eq. (4.12) represents that A (gum acacia), B (gum xanthan) and interactions terms B^2 & AB^2 shows positive influence on the Water Vapor Permeability of the biopolymeric films. The results established by ANOVA (Table 4.3) obtained F-value= 7.04, p-value= 0.0137 and R^2=0.91 for the Water Vapor Permeability and there were no interaction terms found significant effect on the biopolymeric film's water vapor permeability. The influence of A and B on response (Water Vapor Permeability) also found non-significant.



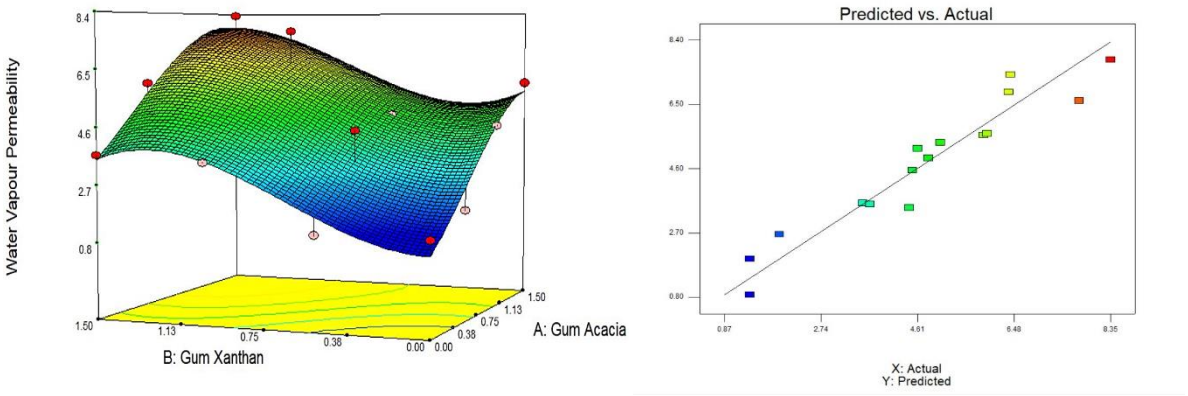
(a) Soubility



(b) Tensile Strength



(c) Elongation



(d) Water Vapour Permilability

Fig. 4.1. 3D plot and Actual v/s predicted linear plot of (a) soubility (b) tensile strength (c) elongation at break and (d) water vapour permilability of the biopolymeric films developed with oilseed meals-gums

Table 4.3. Regression coefficients of the response variables and analysis of variance of the cubic models[a] of oilseed meals-gums (AG: XG) biopolymeric films

Coefficients	Response variables							
	Sum of squares (Y1)	p-value (Y1)	Sum of squares (Y2)	p-value (Y2)	Sum of squares (Y3)	p-value (Y3)	Sum of squares (Y4)	p-value (Y4)
Model	343.25	0.0002	0.0004	0.0243	246.76	0.0218	58.32	0.0137
A-Gum Acacia	4.28	0.0983	0	0.1648	11.41	0.1698	2.90	0.1261
B-Gum Xanthan	1.13	0.3545	8.90×10^{-07}	0.7366	59.62	0.0118	4.74	0.0636
AB	24.39	0.0034	3.02×10^{-07}	0.8441	0.0515	0.92	0.06	0.8016
A^2	78.27	0.0002	0.0002	0.0011	13.71	0.1381	1.44	0.2575
B^2	0.95	0.3923	5.07×10^{-07}	0.7993	7.06	0.2658	0.00	0.9721
A^2B	18.06	0.0070	4.51×10^{-07}	0.8103	52.5	0.0155	0.11	0.7402
AB^2	0.66	0.4720	8.72×10^{-07}	0.7392	15.57	0.1182	0.71	0.4133
A^3	1.48	0.2946	0	0.0484	33.06	0.0378	0.19	0.6670
B^3	1.87	0.2439	2.71×10^{-06}	0.5614	65.03	0.0098	1.03	0.3307
R-Squared	0.98		0.89		0.89		0.91	
Adjusted R^2	0.95		0.73		0.74		0.78	
Residual	6.72		0.0000		28.13		5.52	
Press	108.41		0.0002		328.59		56.31	

[a]$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_{12} AB + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{12} A^2 B + \beta_{21} AB^2 + \beta_{10} A^3 + \beta_{20} B^3$
[b]Coefficient of determination.
Y1= solubility, Y2= tensile strength, Y3= elongation at break and Y4= water vapour permeability

4.3.2. Characterization of oilseed meals-gums (AG: XG) biopolymeric films

4.3.2.1. Solubility of oilseed meals-gums (AG: XG) biopolymeric films

The property of solubility of water in films have critical importance to quantify the volume uptake of water expanding in the length fraction. The property describes the mass absorption of water in film when dipped for 24 h. Table 4.3 presents the values of "Prob > F" less than 0.05, indicating that the model is significant based on the F-value of 0.0002. Table 4.4 displayed the solubility findings, which varied between 29.63% to 45.18%, and were found to be lower than the 47.67% control film, which had no gums. The solubility was seen to decrease with addition of both Acacia and Xanthan gums. The lowest solubility with 29.63% was found in film with 0:1.5 of AG: XG whereas the solubility was found maximum (45.18%) in film with 0.5:0 of AG and XG respectively. Similar results were observed in literature of Dash et al. (2021). The study on flaxseed-alginate biopolymeric film infused with silver nanoparticles has solubility in range of 53.45% to 44.19%. Fig. 4.1 (a) shows the graphical representation of natural gums vs solubility. According to the findings, the solubility of the oilseed meals-gums film is reduced when natural gums are added to oilseed meals film.

4.3.2.2. Tensile strength of oilseed meals-gums (AG: XG) biopolymeric films

According to Rao et al. (2010), the mechanical characteristics of the packing materials aid in determining their ability to tolerate stress applied by external environments or barriers. With a result of 0.0243, the p-value model was determined to be less than 0.05 significant. Table 4.4 shows the tensile strength data for 16 produced films, which varied from 1.007 to 1.026 MPa. The film formed with 0.5:1 AG and XG had the maximum mechanical strength, measuring 1.026 MPa; this was higher than the control film's tensile strength of 1.012 MPa. Whereas, the tensile strength was found least with 1.007 MPa with 0:1.5 AG: XG combination. Protein network incorporated with high amount of natural gum such as gum acacia increases the elongation and decreases the tensile strength as the intermolecular force between macromolecules get weaken due to molecules mobility (Li et al., 2015). In Fig. 4.1 (b), the biopolymeric film is graphically plotted in relation to tensile strength. As a result of the increased xanthan gum content, the oilseed meals-gums biopolymeric film's tensile strength increased.

4.3.2.3. Elongation of the oilseed meals-gums (AG: XG) biopolymeric film

The packing material's elongation at break indicates its flexibility. Alongside tensile strength, the packing material's elongation was investigated. The length difference between the films' original length and the length before the film rupture under test is known as the elongation at break. Indicating that the model was deemed significant with a value of "Prob > F" less than 0.05, the findings of elongation at break were Model F-value 0.0218. The elongation at break presented in Table 4.4 ranges from 5.62% to 20.64%. The films with maximum elongation at break had gum combination of 1.5:1.5 AG and XG whereas the film with least elongation at break had gums combination of 1.5:1 AG and XG. Protein network incorporated with high amount of natural gum such as gum acacia increases the elongation and decreases the tensile strength as the intermolecular force between macromolecules get weaken due to molecules mobility (Li et al., 2015). Similarly, elongation at break explains that the oilseed meals-gums film with highest amount of acacia gum and xanthan gum, had highest value of elongation at break (Fig. 4.1 c).

4.3.2.4. Water Vapour Permeability of oilseed meals-gums (AG: XG) biopolymeric films

When examining the biopolymeric film's water vapour permeability, thickness and starting moisture content are crucial factors to consider. When food products with high moisture content are present, the water vapour permeability of the film helps to stabilise the quantity of water vapour transfer rate. Natural gums and proteins have the same hydrophilic quality that allows them to hold onto moisture (Nandane and Jain, 2018). With the addition of natural gums, the thickness of the biopolymeric films was shown to increase. The film had a thickness that varied from 0.13 mm to 0.51 mm.

Model F-value for water vapour permeability was found to be 0.0137. Table 4.3 presents model terms considered significant based on values of "Prob > F" less than 0.05. Table 4.4 displays the water vapour permeability values of the biopolymeric films, which varied from 1.36×10^{-10} $gs^{-1}m^{-1}Pa^{-1}$ to 8.35×10^{-10} $gs^{-1}m^{-1}Pa^{-1}$. The control film with no added natural gums and least value with gum in ratio (0:0.5) of AG and XG was found similar depicting no such effect was observed. The water vapour permeability was found highest in film with gum combination (1.5:1.5) as AG and XG. This shows that with addition of gums increases the permeability in the films due to high moisture absorption. Conjugating the polysaccharides facilitates protein mobility which maximize the

loosening effect in protein films to trap high moisture, thus high amount of gum addition increases the water vapour permeability (Li et al., 2015).

The water vapour permeability of the oilseed meals-gums biopolymeric films is explained in Fig. 4.1 (d). The films' thickness has a major impact on the water vapour permeability findings. When natural gums are added, the films get thicker. The water vapour permeability of the thickest oilseed meals-gums film was observed to be the greatest.

Table 4.4. Responses of the dependent and independent variables for the oilseed meals-gums (AG: XG) biopolymeric films prepared with different concentrations.

Run	Gum Acacia (%)	Gum Xanthan (%)	Solubility (%)	Tensile Strength (MPa)	Elongation at break (%)	Water Vapour Permeability×10^{-10} ($gs^{-1}m^{-1}Pa^{-1}$)
1	1.5	1	33.25	1.018	5.62	6.38
2	0	0	47.67	1.012	8.31	1.36
3	1	0.5	40.98	1.022	18.50	4.82
4	0.5	1	41.25	1.026	12.36	4.61
5	0.5	1.5	37.8	1.021	13.73	5.96
6	0.5	0.5	42.55	1.020	13.33	4.45
7	0.5	0	45.18	1.021	10.74	1.93
8	1	1.5	36.43	1.018	16.37	6.41
9	0	1.5	29.95	1.007	18.85	3.69
10	1	0	42.59	1.021	16.64	4.51
11	0	0.5	38.5	1.010	14.79	1.36
12	1.5	1.5	31.63	1.015	20.64	8.35
13	0	1	34.98	1.009	12.85	3.55
14	1.5	0.5	35.63	1.015	11.50	5.05
15	1	1	39.34	1.016	12.32	7.74
16	1.5	0	39.13	1.017	6.62	5.89

Note: GA= Gum Arabic, GX= Gum Xanthan, TS= Tensile Strength, EAB= Elongation at break and WVP= Water Vapour Permeability

4.3.2.5. Optimization of gums (AG: XG) concentration

In order to investigate the mechanical and barrier qualities of the oilseed meal/natural gum biopolymeric film, a complete factorial design was used to achieve the

best results. The use of natural gums enhanced the blended oilseed meal film's composition. Optimising the amount of natural gums required to be added to the oilseed meal film-forming suspension involved the use of a full factorial design. It was determined that the ideal blend ratio for biopolymeric film formulation was 0.5:1.5 AG:XG, based on the way the independent variables (natural gums) interacted with the responses. To validate to actual results v/s the predicted values, the film formulation was tested in triplicate. The results were found similar as shown in Table 4.5. This imploy that the developed (0.5:1.5 AG:XG) oilseed meals-gums film had acceptable barrier, physical and mechanical properties that can be applied for further practical utilisation. The production conditions for a successful biopolymeric film required a minimal solubility, a minimum water vapour permeability & elongation at break with maximum tensile strength.

Overall findings for oilseed meals-gums biopolymeric films with gum combination were related to the optimised film condition (Fig. 4.2). The validation of optimization with graphical representation of overall properties of the developed oilseed meals-gums (0.5: 1.5 AG: XG) biodegradable film. The graph indicates the region of the optimal progress variable setting of the design. The model values indicating the flag shows 0.609% desirability advising well representation of responses as function of different factors (Table 4.5). The graphical representation of acacia gum and xanthan gum interaction (ranges 0-1.5) intercepting a point shows desirable point of gums combination acceptable for film development. For illustration, linear plot comparison of model predicted responses v/s the experimental responses (solubility, tensile strength, elongation at break and water vapor permeability) in Fig. 4.1 (a-d) also implying a close association and good model fitness.

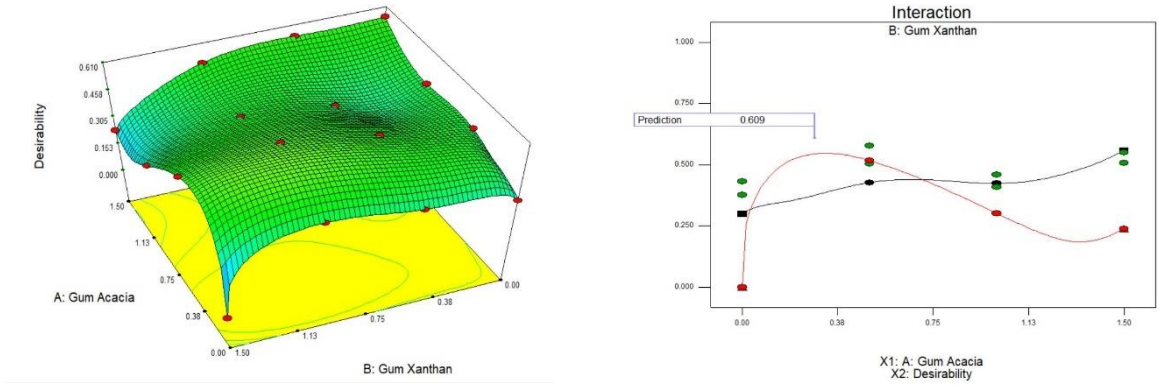


Fig. 4.2. 3D plot and 2D interaction representation of overall properties of the biopolymeric films developed with oilseed meals-gums.

Table 4.5. Recommended and experimental solutions for the optimized oilseed meals-gums (AX: XG) biopolymeric film suspension

GA	GX	Y1	Y2	Y3	Y4	
0.5	1.5	38.43	1.02	9.23	4.76×10^{-10}	Predicted
		41.84	0.98	9.21	4.43×10^{-10}	Experimental

GA = Gum Arabic, GX = Gum Xanthan

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

4.4. Results and Discussion

b) To study the effect of crosslinkers (citric acid and glutaraldehyde) on the properties of the biopolymeric films

4.4.1. Effect of addition of crosslinkers (Citric Acid and Glutaraldehyde) on properties of oilseed meal and gums based biopolymeric films

The biopolymeric films developed from oilseed meals in combination with natural gums were studied. The best biopolymeric film was selected was (0.5:1.5 AG:XG) oilseed meals-gums film were taken as control biopolymeric film named as OG for (oilseed meal-gums film) for further study and OG (oilseed meals-gums film) was crosslinked with two types of crosslinkers named as Citric Acid and Glutaraldehyde. The concentration of the crosslinkers were taken in percentage (w/w) of the oilseed meal sample weight. The design of the film formulation is shown in Table 4.2. Table 4.6 was helpful in studying the development of the best compactable crosslinker for oilseed meals-gums crosslinkers blended biopolymeric films and discusses about the comparative study of the Citric Acid and Glutaraldehyde crosslinkers and their effect on the biopolymeric film formulation.

4.4.1.1. Thickness and Moisture content

Thickness and moisture content play an important factor in the film development. The mechanical properties depend on the thickness of the film whereas, moisture content should be sustained to increase the shelf of the film as well as the food preserved in it. The thickness of the oilseed meals-gums crosslinked biopolymeric films ranged from 0.46±0.02 to 0.56±0.03 mm. The oilseed meal-gums crosslinked film with highest thickness and lowest in thickness is found in film named as CA-film 4% and GL-film 10%

respectively. The values of thickness varied significantly after addition of crosslinkers to the oilseed meals-gum control films.

The moisture content of the film is shown in Table 4.6. The film with lowest moisture content was found in oilseed meals-gums crosslinked film with glutaraldehyde at 10% (GL-film 10) and the highest moisture content was found in oilseed meals-gums crosslinked film with glutaraldehyde at 2% (GL-film 2). The control film named as OG (oilseed meals-gums) without crosslinkers had moisture content with 33.41±1.82%. The reading of the crosslinked film did differ significantly from that of the control (oilseed meals-gums) film. With increased inclusion of both crosslinkers (CA & GL), a reduction in moisture content was seen. Similar trend was seen by Seligra et al. (2016) when citric acid was added starch-glycerol eco-films. The proposed the reason of decrease in moisture content was due to free OH groups which supplemented intermolecular hydrogen bonds with starch, hence water resistibility. Here, the OH bonds must have conjugated with proteins available in films.

4.4.1.2. Solubility of oilseed meals-gums crosslinked (CA & GL) biopolymeric films

The results of water solubility of the biopolymeric crosslinked films are shown in Table 4.6. It can be observed clearly that in both cases of crosslinking, the water solubility of the films drastically decreases as the concentration of the crosslinkers (CA & GL) is increased. The water solubility was found highest in oilseed meals-gum crosslinked film with 2% citric acid in comparison with control oilseed meals-gums film. There was significant difference observed in the values of control oilseed meal-gum films and crosslinked films. Similar case study was observed in soybean residue based edible film crosslinked with Citric acid. This dramatic effect of citric acid on water solubility was due to insoluble fibres present in oilseed meals binding (Ma et al., 2018).

4.4.1.3. Tensile strength

Table 4.6 displays the comparison results of the mechanical properties of the two crosslinkers (CA & GL) that were added to the oilseed meals-gums biopolymeric films. Comparing the two crosslinked films, oilseed meals-gums (AG: XG) crosslinked (CA) & oilseed meals-gums (AG: XG) crosslinked (GL) to the control film, oilseed meals-gums (OG), a noticeable improvement in tensile strength was noted after crosslinking. Oilseed meals-gums crosslinked film that was crosslinked with 10% citric acid had the highest tensile strength with 1.15 MPa, while oilseed meals-gums crosslinked film that contained

2% glutaraldehyde had the lowest with 0.66 MPa. It was observed that with increase in addition of crosslinkers, the tensile strength increased. The values also found significantly different after crosslinking the films. Similar impact of glutaraldehyde crosslinking of chitosan film was observed by Pavoni et al. (2021). Citric Acid also increased the mechanical property of neem seed oil meals based film due to creation of crosslinking between the polymeric chains thus, the strength and modulus increases (Aramwit et al., 2022).

4.4.1.4. Percent Elongation

Elongation at break property of the crosslinked tends to decrease in comparison to the tensile strength of any film (Aramwit et al., 2022). Similar observation was recorded in case of the control oilseed meals-gums biopolymeric film when added with crosslinkers (CA & GL). As the crosslinkers percentage increased, the elongation of the oilseed meals-gums crosslinked film increased but decreased eventually. The elongation was found in the range of 9.69% to 13.10%. The elongation was highest in oilseed meals-gums crosslinked film with 6% Citric acid and was found lowest in oilseed meals-gums crosslinked film with 6% Glutaraldehyde. There was significant difference in between the values of oilseed meals-gums and oilseed meals-gums crosslinked films. Citric acid has the property of cross-linking with hydroxyls of polysaccharide, thus improves its mechanical strength and improves the compatibilization between polymeric molecules of films (Ma et al., 2018). Lower elongation results from crosslinking because it strengthens and modifies the polymer chains, preventing free movement and limiting mobility. There's also a chance of excessive crosslinking, also known as "over crosslinking" which results in a significant reduction in elongation. The result of elongation property comparison between two different crosslinkers (CA & GL) added in the oilseed meals-gums crosslinked biopolymeric films are shown in Table 4.6.

4.4.1.5. Water Vapour Permeability

Table 4.6 displays the water vapour analysis of the oilseed meals-gums crosslinked films. According to the results, the water vapour permeability of the oilseed meals-gums crosslinked films improved (decreased) when compared to the control films oilseed meals-gums without crosslinkers. The permeability tends to decrease as the oilseed meals-gums crosslinked films as crosslinker percentage increased. The water vapor permeability reading of oilseed meals-gums crosslinked films ranges from 3.07 $gs^{-1}m^{-1}Pa^{-1}$ to 4.32 gs^{-}

$^{1}m^{-1}Pa^{-1}$ found highest in 2% citric acid crosslinked film and lowest in 10% glutaraldehyde crosslinked films. The values were found significantly different. The soy protein isolate was found to have a similar effect when blended with cod skin gelatin films and zein in the protein matrix. This was attributed to the hydrophilic sites in the film matrix being hindered, which allows for higher diffusion of water molecules and, ultimately, higher water vapour permeability (Chambi and Grosso, 2006; Denavi et al., 2009; Fan et al., 2018).

Table 4.6. Comparative responses of the dependent and independent variables for the oilseed meals-gums, oilseed meals-gums citric acid crosslinked and oilseed meals-gums glutaraldehyde crosslinked biopolymeric films prepared with different concentrations.

Biopolymeric films nomenclature		Moisture content (%)	Thickness (mm)	Solubility (%)	Tensile Strength (MPa)	Elongation at break (%)	Water Vapour Permeability× 10^{-10} ($gs^{-1}m^{-1}Pa^{-1}$)
oilseed meals-gums	OG	33.41±1.82^{bc}	0.49±0.03^{abc}	41.84±1.17^{cd}	0.98±0.22^{abc}	9.21±1.96^{a}	4.43±0.010^{g}
Oilseed meals-gums Citric Acid Films	OGCF 2%	34.65±1.29^{c}	0.54±0.02^{cd}	42.92±1.15^{d}	0.85±0.10^{abc}	11.94±2.30^{ab}	4.32±0.009^{g}
	OGCF 4%	33.28±2.26^{bc}	0.56±0.03^{bcd}	40.62±0.66^{cd}	0.88±0.14^{abc}	11.10±1.24^{ab}	4.18±0.005^{f}
	OGCF 6%	32.51±1.14^{bc}	0.54±0.02^{cd}	39.24±0.52^{bc}	0.98±0.09^{abc}	13.10±0.86^{b}	4.16±0.004^{f}
	OGCF 8%	31.00±2.29^{ab}	0.54±0.01^{cd}	38.76±0.61^{cd}	1.06±0.27^{bc}	11.46±0.88^{ab}	4.15±0.074^{f}
	OGCF 10%	30.98±1.00^{ab}	0.55±0.01^{cd}	35.83±0.37^{a}	1.15±0.30^{c}	10.68±0.83^{ab}	3.8±0.010^{d}
Oilseed meals-gums Glutaraldehyde films	OGGF 2%	35.39±0.13^{c}	0.50±0.02^{abc}	41.14±0.42^{cd}	0.66±0.16^{a}	9.70±2.49^{a}	3.91±0.042^{e}
	OGGF 4%	34.86±0.66^{c}	0.48±0.02^{ab}	39.60±2.78^{bc}	0.74±0.04^{ab}	12.40±0.30^{ab}	3.94±0.040^{e}
	OGGF 6%	33.86±1.47^{bc}	0.48±0.04^{bc}	37.84±0.61^{ab}	0.82±0.12^{abc}	9.69±0.57^{a}	3.92±0.040^{e}
	OGGF 8%	33.73±0.85^{bc}	0.51±0.03^{ab}	36.92±1.06^{ab}	0.91±0.10^{abc}	12.52±1.27^{ab}	3.66±0.038^{c}
	OGGF 10%	28.35±1.35^{a}	0.46±0.04^{a}	36.03±1.34^{a}	0.92±0.11^{abc}	11.67±0.92^{ab}	3.07±0.042^{a}

OGCF= oilseed meals-gums films with citric acid, OGGF= oilseed meals-gums film with glutaraldehyde & OG= oilseed meals-gums film without crosslinkers. All the mentioned values are means of three replicates ± standard deviation. The letters superscripted as a, b, c & d on the values shows significant differences ($p< 0.05$).

4.4.1.6. Selection of crosslinker for oilseed meals-gums crosslinked film formulation

The comparative study of the oilseed meals-gums crosslinked film added with Citric acid and Glutaraldehyde was carried out as shown in Table 6. The use of both crosslinkers improved the properties of the oilseed meals-gums crosslinked films in comparison to the oilseed meals-gums film. It was determined that the ideal crosslinker for biopolymeric film formulation was with 10% Citric acid was found more suitable. The oilseed meals-gums crosslinked films was applied for further sophisticated analysis such as Color, DSC, FTIR, SEM and XRD in comparison with oilseed meals-gums film.

4.4.2. Properties of oilseed meals and gums based film crosslinked with citric acid

4.4.2.1. Colour

Using the Hunter Lab colorimeter (Ultrascan VIS, HunterLab. Inc., USA) in reflectance mode, a comparative examination of the colour analysis of the oilseed meals-gums and oilseed meals-gums crosslinked films was conducted with respect to L*, a*, b*, and whiteness index. The whiteness of the film is determined by whiteness index in which if the scale indicates higher, the lighter is the color of the film (Lee et al., 2016). The values of L*, a* and b* for both biopolymeric films are presented in Table 4.7. The whiteness index of the oilseed meals-gums crosslinked film was found higher with 21.27 than oilseed meals-gums film with 20.53. Similarly, L*, a* and b* values of oilseed meals-gums crosslinked film were also found higher than oilseed meals-gums film. For oilseed meals-gums crosslinked film, the corresponding values of L*, a*, and b* were 25.38±0.82, 3.03±0.42, and 3.11±0.20. Additionally, the oilseed meals-gums film's L*, a*, and b* values were 24.38±0.36, 0.58±0.05, and 1.15±0.09, respectively. The result shows that the L* value of both films is less than 30, i.e. the values are nearer to 0, so the films are visually darker in color.

Both the biopolymeric films have positive 'a' and 'b' values, i.e., the films are visually the combination of redness and yellowness. In comparing the oilseed meals-gums film and oilseed meals-gums crosslinked film, the film after crosslinking got darker in shade. There was slight increase in the values of L*, a* and b* after the addition of citric acid in the film. Similar case was observed in the edible films of soybean residue added with citric acid. The reason behind this can be change in the native structure of film as structurally film matrix is bound with external extract or addition of phenolic compound

(Kadam and Lele, 2018). Usually, the films made from oilseed meals such as mustard and flaxseed are darker in color than the other oilseed meals. This also affect the color properties of oilseed meals-gums crosslinked films. Rasel et al. (2016) also found similar results in case of the packaging films developed from Crambe abyssinica/gluten.

Table 4.7. Color parameters (L*, a*, b*), Whiteness Index and Thermal properties of the oilseed meals-gums in comparison to oilseed meals-gums citric acid crosslinked biopolymeric films.

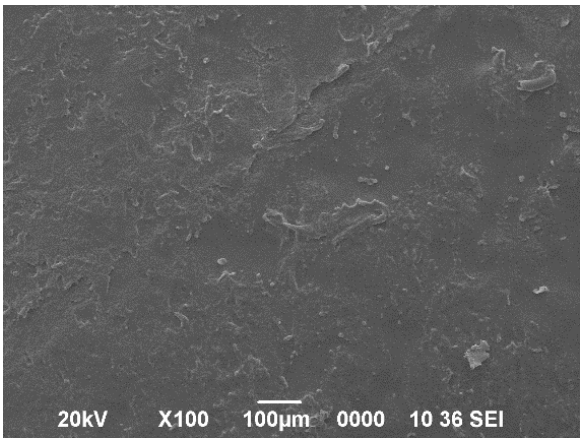
	Glass Transition temperature (°C)	Melting range (°C)		Melting peak (°C)	Melting Enthalpy (J/g)	Colour Analysis			
Biopolymeric films	**T_g**	**T_o**	**T_e**	**T_m**	**ΔH_m**	**L***	**a***	**b***	**WI**
Oilseed meals-gums film	112	173	246	218	20.93	24.38±0.36	0.58±0.05	1.15±0.09	20.53
Oilseed meals-gums crosslinked films	115	185	252	232	15.51	25.38±0.82	3.03±0.42	3.11±0.20	21.27

All the mentioned values are means of three replicates ± standard deviation.

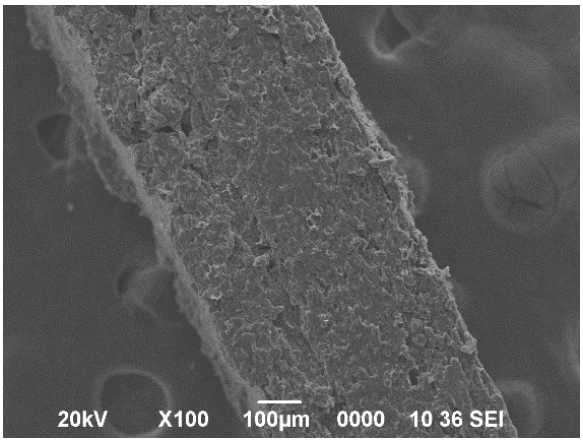
4.4.2.2. Scanning electron microscopy (SEM)

Fig. 4.3 depicts the cross-sectional and surface microstructure of the oilseed meals-gums and oilseed meals-gums crosslinked films. When compared to oilseed meals-gums crosslinked film, the oilseed meals-gums films' surface morphology is significantly different, as shown by SEM analysis. The oilseed meals-gums film is smoother in display than the oilseed meals-gums crosslinked film. There is presence of binding in pores and between the cells can be seen in the oilseed meals-gums crosslinked films. The cross-sectioned microstructure showed no significant difference between the oilseed meals-gums films and oilseed meals-gums crosslinked film. Similar study on edible soybean residue based film added with citric acid found that the surface of the film became smoother with increase in the percentage addition of citric acid (Ma et al., 2018). Additionally, the study conducted by Li et al., (2015) on peanut protein isolate-gum Arabic produced films revealed that the films' surfaces were smooth, compact, and had few holes

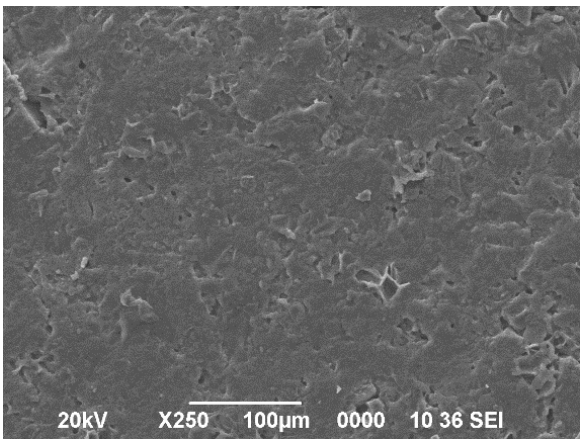
or fractures. In contradictory, the pure xanthan gum films developed uneven surface in films due to its high viscosity of xanthan gum and irregular distribution of gel on petri plates (Mohsin et al., 2020).



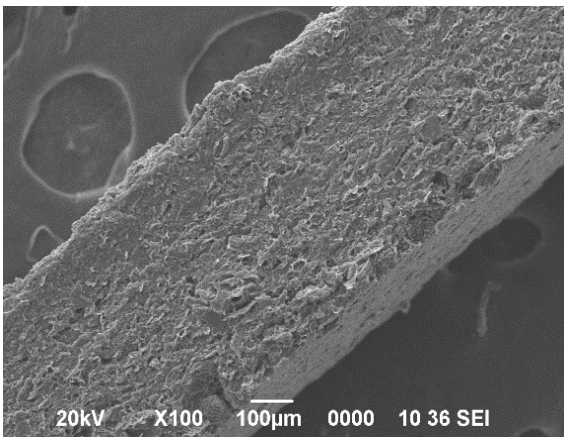
(A) Oilseed meals-gums film (surface)



(B) Oilseed meals-gums film (cross-section)



(C) Oilseed meals-gums /crosslinked film (surface)



(D) Oilseed meals-gums /crosslinked film (cross-section)

Fig. 4.3. Scanning Electron Micrographs at 100 × magnification of the surface (A & C) and cross-section (B & D) of the biopolymeric films developed with oilseed meals-gums as well as added with citric acid as crosslinker.

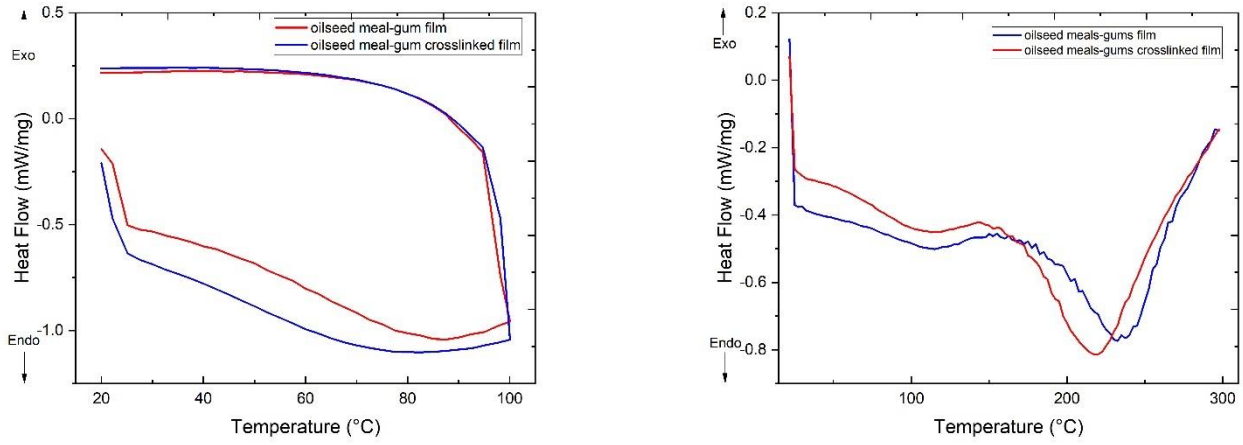
4.4.2.3. Differential scanning calorimetry (DSC)

The impact of the film's thermal characteristics was examined using DSC analysis. Film processing benefits from both the mechanical characteristic and the ability to adapt to varying heat ranges (Kadam and Lele, 2018). A material when are subjected to heating and cooling cycles within a certain temperature range, the amount of energy stored and

released can be determined using DSC. DSC also describes the melting enthalpy and crystallization enthalpy describes the amount of energy stored and released by the material (Kizildag, 2023).

The graphs plotted for oilseed meals-gums and oilseed meals-gums crosslinked films are presented in Fig. 4.4 (a & b). The DSC analysis on the biopolymeric films was performed in two-phase. In the first heating phase, the temperature range was kept from 20ºC to 100 ºC and then cooled at temperature range from 100 ºC to 20 ºC. In the 2nd heating phase, the temperature was raised from 20 ºC to 300 ºC. The graphs were plotted according to the heating stage as shown in Fig. 4.4 and Table 4.7 lists the equivalent phase transition temperatures and enthalpy values, including glass transition temperature (Tg), melting enthalpy (ΔHm), onset temperature (To), end temperature (Te), and melting temperature (Tm). There was absence of any phase transition in the continuous 1st heating and cooling stage at temperature range between 20 ºC to 100 ºC as show in Fig. 4.4 (a & b) and no significant difference was observed between the graphs of oilseed meals-gums film and oilseed meals-gums crosslinked film.

In the 2nd heating stage of the films, the initial path covered was recorded similar to 1st heating stage in the graph. This indicates the regaining of the structure of the film in cooling stage between 100 ºC to 20 ºC. The glass transition temperature (T_g), onset melting temperature (To), melting end temperature (Te), melting peak (Tm) and enthalpy of melting (ΔH_m) of oilseed meals-gums film (Table 4.7) were 112 ºC, 173 ºC, 246 ºC, 218 ºC and 20.93 ºC respectively. Whereas, the glass transition temperature (T_g), onset melting temperature (To), melting end temperature (Te), melting peak (Tm) and enthalpy of melting (ΔH_m) of oilseed meals-gums crosslinked film were recorded as 115 ºC, 185 ºC, 252 ºC, 232ºC and 15.51 ºC respectively. In comparison, DSC of both film values clearly shows there was decrease in the thermal values of film. The decrease in T_g can be due to better crosslinking between molecules of oilseed proteins and gums after crosslinking with citric acid. Decrease in glass transition temperature is due to change in degree of crystallinity of the film and the presence of higher moisture content during the analysis (Kadam and Lele, 2018; Li et al., 2018).



(a) (b)

Fig. 4.4. Differential scanning calorimetry (DSC) curve of (a) 1st heating phase (20 ºC-100 ºC) & cooling phase (100 ºC to 20 ºC) and (b) 2nd heating phase (20 ºC to 300 ºC) the biopolymeric films developed with oilseed meals-gums as well as added with citric acid as crosslinker.

4.4.2.4. Fourier-transform infrared spectroscopy (FTIR)

The Fourier-transform infrared spectroscopy is valuable in revealing the molecular characterisation and chemical reactions between the polymers used in film formation. The FTIR analysis of the biopolymeric films prepared of oilseed meals films, oilseed meals-gums and oilseed meals-gums crosslinked were shown in Fig. 4.5. The patterns of both biopolymeric films in graph obtained were not significantly different from each other since the basic elements of the film are common. There were peaks present in oilseed meals-gums films that seem to resolve after crosslinking in oilseed meals-gums crosslinked films. Apart from common peaks, there were additional new peaks found after crosslinking. A broad spectrum was observed in the wavenumber range 3500-3000 cm^{-1} at 3424 cm^{-1} characterizing -OH group from gums and moisture content present in film but tends to broaden after crosslinking showing results of chemical interactions between the blended polymers (Li et al., 2015). The peak at 1729 cm^{-1} between range of 1730-1715 cm^{-1} was appeared to be the presence of citric acid assisted as C=O strong stretching or formats α, β- unsaturated ester groups between citric acid and glycerol suggesting development of crosslinking (Seligra et al., 2016). The vibrations found between 1500-1550 cm^{-1} showed a strong N-O (amine II) stretching nitro compound related to traces of proteins and the range at 2800-3000 cm^{-1} showing N-H stretching amine salt (amide III) (Li et al., 2015).

Both the graphs show common strong peaks at 1651 cm-1, 1109 cm^{-1}, 1041 cm^{-1}, 851 cm^{-1} and 675 cm^{-1} showing the presence of C=C medium stretching alkene vinylidene, C-O aliphatic ether stretching, CO-O-CO strong anhydride bond, C-Cl stretching and C-Br stretching of strong halo compound respectively which were found similar to earlier chapters with little shifts in range.

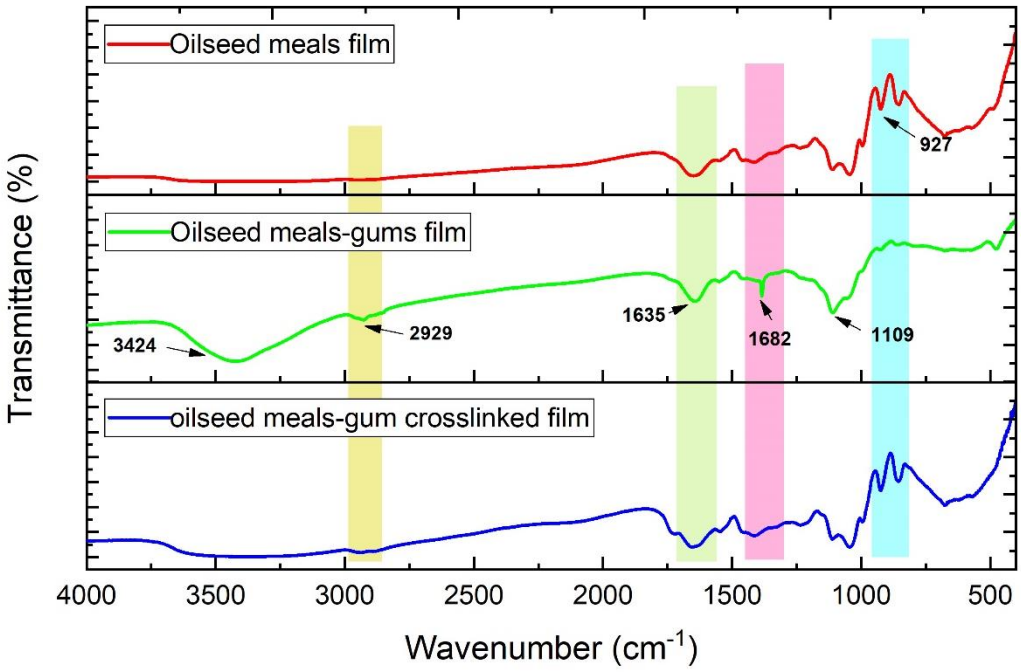


Fig. 4.5. Fourier-transform infrared spectroscopy graph of the biopolymeric films developed with oilseed meals-gums as well as added with citric acid as crosslinker.

4.4.2.5. X-ray diffraction

X-ray diffraction is useful in determining the degree of crystallinity and the crystalline phase of the biopolymeric films. The characterization of XRD of any sample is of its sharp peaks correlated to crystalline diffraction and the amorphous zone in the graph. The pattern of crystalline for oilseed meals films, oilseed meals-gums and oilseed meals-gums crosslinked film with citric acid are presented in Fig. 4.6. There was presence of major sharp peaks in oilseed meals film at 21.07º and 27.01º which found to shift in oilseed meals-gums film at 20.37º, 26.16º and 30.45º. Similar peaks were found in the film prepared from gum acacia and xanthan gum based films as described in literature of Kurt et al. (2017); Nair et al. (2020). Whereas the peaks broadened and shifted to 19.77º, 20.27º,

26.16º and 27.55º in the oilseed meals-gums crosslinked films. The broadened peaks at 2θ =20º indicated that the film is in amorphous state and there is presence of intermolecular interaction, and outstanding miscibility among the molecules of proteins of oilseed with gum and crosslinkers. Mixing of polysaccharides with polymers of protein reduces the crystalline structure to amorphous phase thus, interfering with protein chains arrangement (Li et al., 2015). The shifting the peaks after crosslinking also describes the shrinking or expansion of cell units in ionic radius that leads to the peak position, peak broadening or intensity of peaks (Nair et al., 2020). Oilseed meals-gums film had crystallinity percentage of 43.21%, whereas oilseed meals-gums crosslinked with citric acid film had a percentage of 36.06%.

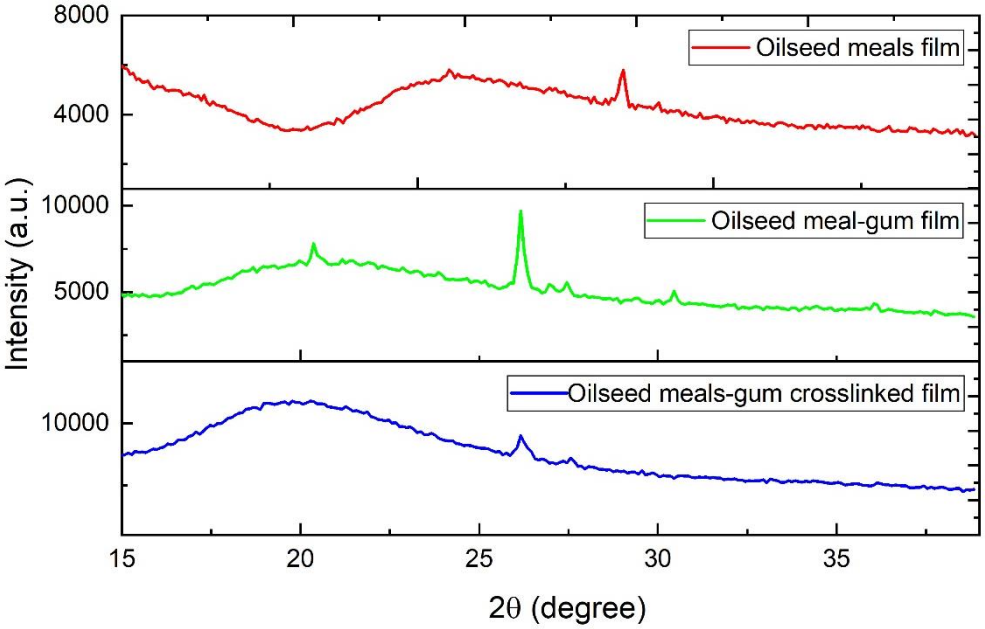


Fig. 4.6. X-ray diffraction graph of the biopolymeric films developed with oilseed meals-gums as well as added with citric acid as crosslinker.

4.5. Conclusions

A cubic model was produced using the full factorial design, which was shown for every respondent and further experimentally confirmed, showing that the blended biopolymeric film of soybean meal and natural gums had a substantial impact on many film characteristics. It was determined that 0.5: 1.5 AG: XG blended suspension had overall the most accepable compactability with the oilseed meals. The film emulsion with gum exibited more stable polymer network than the oilseed meal based films. The oilseed meals-gums crosslinked films were also successfully developed after incorporation of crosslinkers into oilseed meals-gums suspension. It was found that the films crosslinked with 10% Citric acid (CA-film 10) was found to have better results in comparison to the films crosslinked with 10% Glutaraldehyde (GL-film 10). Citric acid being non-toxic, economic and safer for food packaging is more acceptable as crosslinker than Glutaraldehyde that can be continued for further studies.