Development of sugar free cookies with novel biodegradable packaging film

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INTRODUCTION

India has been recently declared as the ‘diabetic’ capital of the world. One in every five persons in India suffers from diabetes and other related disorders (Joshi et al., 2005). According to the International Diabetes Federation (Diabetes Atlas ninth edition, 2019) an estimated 463 million (9.3%) adults aged 20–79 years are currently living with diabetes. This number is predicted to rise to 578 million (10.2%) by 2030 and to 700 million (10.9%) by 2045. People suffering from diabetes are advised to restrict their use of sugars. (Aggarwal, et al., 2016) The use of alternative sweeteners can help manage weight and normal blood glucose level (Deshmukh, et al., 2019).

Cookies have gained popularity amongst consumers due to their ease in availability, affordability, varietal flavours, and light texture. They are shelf-stable, ready to eat, wholesome, nutritious snacks with enhanced value addition. However, due to the growing health consciousness of the consumers, foods containing high amount of sugar and fat with high glycemic index (GI) may predispose them to overweight and obesity. (Handa et al., 2012). Nowadays, cookies made from oats are much in demand because of their enhanced functional properties. Oats are rich in dietary fibre, specifically, β-glucan, which is a soluble fibre, known to reduce blood cholesterol level by increasing the excretion of bile in the body (Jenkis and Kendal 2002). In addition to this it is also high in proteins, minerals such as iron, zinc and vitamin B3.

How to Cite


Abstract

The use of alternative sweeteners can help manage weight and normal blood glucose levels of diabetics. Development and standardization of sugar free, low glycemic index and high fibre cookies using wheat flour, oats, trans free bakery shortening, and almonds. Physiochemical analysis of the raw materials used for cookie preparation and the finished product was conducted. Cookies were analysed for diameter, height, spread ratio, texture, and water activity. Sensory analysis using semi-trained panellists was done to establish the acceptability of the product. The formulated cookies were well accepted by the semi-trained panellists as well as the people with diabetes who were randomly selected for the study. The overall appearance, texture and flavour of the cookies were moderately liked by the panellists as indicated in the qualitative descriptive analysis. The product did not change much with the storage of 90 days. The cookies were high in dietary fibre (2.5g per serving), out of which β-glucan, a soluble fibre was found to be 0.8g per serving which offers a healthy alternative for consumers. The biodegradable polymer used for packaging the cookies was prepared using terpolymer. The chemical and physical properties of the polymer were determined using acid value which was between 0.0195 and 0.0200, hydroxyl value; 0.0260 and 0.023 and the molecular weight was in the range of 10,256 δ and 10,000 δ of the terpolymer A and B, respectively. The polymer demonstrated good mechanical strength as well as water vapours barrier properties to be used as a primary package for cookies.

Keywords: Glycemic Index, High Fiber, Diabetics, biodegradable, packaging
as iron and magnesium and pantothenic acid (B₅).
There is also an increasing demand for products with reduced energy, especially those that use sweeteners as substitutes for sucrose (Trevizian et al., 2010). Substituting sugar with low-calorie sweeteners may be an efficacious weight management strategy.
Sugar plays a significant role in providing golden brown colour, pleasant aroma, crumb structure and overall acceptability to the baked products. But due to the increase in the prevalence of non-communicable diseases at an alarming rate such as diabetes, obesity, dental caries and cardiovascular diseases, nowadays, non-nutritive intense artificial sweeteners are being used as substitutes for developing sugar-free products by the food industry. (Aggarwal, D. et al., 2016) Substitution of artificial sweeteners with sucrose may facilitate the maintenance of nutritionally balanced diet by satisfying a diabetic person’s desire for sweets and assist in the control of calorie intake (Chattopadhyay et al., 2014).
However, the use of artificial sweeteners poses technological challenges to the food processor during baking. The crucial functions of sucrose in foods are not easy to mimic by its replacers. Substitution of sugar with intense non-nutritive artificial sweeteners affects the physiochemical properties of baked products. Partial replacement of sugar in muffins with intense sweeteners along with inulin was done to achieve desired structure and sweetness. (Gao et al., 2018).
Several studies have been conducted to study the effect of sugar substitution with artificial sweeteners on the quality of cookies (Savitha et al., 2008). Sucralose has been reported to have excellent product stability even when subjected to high temperatures during baking. Sugar alcohols have been suggested as bulking agents for the low-calorie baked goods, as intense sweeteners lack texture modifying property like sucrose. Polyols such as sorbitol, maltitol, xylitol etc. have been employed in the manufacture of sugar-free candies, cookies and chewing gums in the industry. (Zoulas et al., 2000). Therefore, the present study was undertaken to provide consumers with healthy food choices and help in the management of non-communicable diseases such as diabetes mellitus, obesity and cardiovascular diseases.
In India, biodegradable polymeric films which are economical as well as eco-friendly are in great demand. Plastic is synthetic polymer which is used in the entire world due to its mechanical properties, long durability and its cost effectiveness but its properties like biodegradation resistant, its inertness and hard to degrade and obvious human behaviour of littering plastic are the major consequences for the need of biodegradable plastic. Most of the plastics in the market claimed to be biodegradable are based on synthetic and microbial polyesters. Different degradable polymers such as polylactides, polyethylene-carbon monoxide polymers (3-hydroxybutyrate-3-hydroxy valerate), vinyl ketone copolymers (Guillet process), and starch-filled polyethylene (Griffin process), have been developed. These plastics differ in degradation rate, application, and price. (Muller et al., 2001) has reported that plastic inertness and resistance to microbial attack was reduced by incorporating starch and pro-oxidants. The aromatic polyesters such as polyethylene terephthalate and polybutylene terephthalate have excellent material properties as compared to most aliphatic polyesters and also their susceptibility to microbial attack is negligible. Therefore, to increase the biodegradability of aromatic polyesters, some studies focused on the synthesis of aliphatic-aromatic copolyesters or incorporation of aliphatic dicarboxylic acids or polyethylene glycol in polyester chains which greatly enhance the degradation rate (Gnanavel et al., 2012).
Among these polymers much progress has been made in polyactic acid (PLA), Polyglycolic acid (PGA) and their copolymers. These can be synthesized in wide range of molecular weights by two methods; through direct condensation reaction of lactic acid/ or glycolic acid which leads to low molecular weight and secondly, by ring opening polymerization of cyclic dimer i.e. lactide and glycolide, in the presence of metal catalyst to synthesize high molecular weight polymers (Lindani et al., 2020). Therefore, in the present study, an attempt was made to prepare a novel thermoplastic polymer using the poly-condensation approach to produce terpolymer of lactic acid, phthalic anhydride and ethylene glycol. This biodegradable packaging material was also studied for tensile strength and water vapour transmission rate.

MATERIALS AND METHODS

Raw material source and analysis
Whole wheat flour and rolled oats were procured from the local Delhi market. Bakery shortenings which were trans-fat free was obtained from Cargill Foods Delhi, India. Sucralose (sugar free – cadillazydus) was selected and purchased on the basis that it is approved for food use by FSSA, 2006 and is heat stable during baking. Vanilla essence, baking powder, almonds were also procured from the local market of Delhi. All raw materials were analysed for physical and chemical quality as per AOAC (1998) procedures to ensure the good quality of the finished products (Table 1).

Physical analysis
Wheat flour and oats were analyzed physically on the basis of its colour, feel/ texture and insect infestation.

Chemical analysis
Determination of moisture content, ash content, water absorption power, gluten content, sedimentation value

317
was done for wheat flour and moisture content and total ash for oats was done, respectively.

**Proximate analysis of cookies**
It included determination of moisture, protein, fat, dietary fibre and ash content.

**Microbiological analysis**
Total Plate Count (TPC) and *E. coli*

**Sensory analysis**

**Cookie formulation**
Short dough cookies were prepared as per the AOAC method (2004) with some modifications by creaming bakery shortening with sucralose a non-nutritive sweetener and vanilla essence until pale and fluffy using planetary mixer (Moulinex, Masterchef compact) at speed 2 for 5 minutes (Table 2). Refined flour was sifted with baking powder and salt and then stirred until well mixed. Rolled oats, almonds were incorporated. Standardized amount of water was added and the dough was kneaded for 5 minutes. The cookie dough was wrapped in plastic wrap to prevent moisture loss and kept in the refrigerator for an hour. The dough was tempered to room temperature (21°C) and scooped with an ice cream scooper before being dropped on the baking sheets to be baked for 30 minutes in a preheated oven at 180°C. The cookies were allowed to cool for 1 hr at ambient temperature. The cookies were stored at room temperature (22-24°C) and sensory and instrumental evaluations were performed.

**Physical evaluation of cookies**

**Cookie diameter, height and spread ratio**
Cookie diameter (cm) was measured by laying six cookies edge to edge with the help of scale and then rotating them by 90° and re-measuring. The average diameter of the cookies was the average of the two readings divided by six. Cookie height (cm) was determined by stacking six cookies on top of one another, restacking and re-measured. The average height of the cookies was the average of the two readings divided by six. Spread ratio, which is defined as a ratio of average diameter to an average height of the cookies, was then calculated.

**Instrumental analysis**

**Cookie texture:** A texture analyser (TA-XT Plus), Stable Microsystems, UK equipped with a 50 kg load cell was used for cookie texture evaluation. Cookies were evaluated for hardness within 24h. The cutting strength was measured using HDP/BS blade of texture analyser. The individual samples of biscuits were placed on the platform and the blade was attached to the crosshead of the instrument. The TA settings selected were pre-test speed: 2mm/s, test speed: 3mm/s, post-test speed: 10mm/s and distance 5mm. The absolute peak force of the resulting curve was considered as the cutting strength of the biscuit (Fig. 1).

**Water activity:** Water activity (A_w) of cookie samples was measured with a water activity meter (Aqua Lab model DTE) Decagon Devices Inc., Pullman, WA. It was calibrated with sodium chloride standard solution (6 molal, at 25°C, A_w = 0.7549, Decagon devices). A 30 mm diameter round plug was punched from the centre of each cookie and crumbled inside the sample cup, and inserted into the metering chamber. The A_w was measured using the chilled-mirror method at sample temperature set to 25 ± 0.2°C. The measurements were replicated three times.

**Sensory acceptability tests**

**Panel training**
Forty five students were randomly selected based on their willingness to participate in the sensory training and study. The panel members were Food Technology students pursuing graduation at Bhaskaracharya College of Applied Sciences, University of Delhi, New Delhi, India. The panellists were between 18-20 years of age and were regular consumers of cookies. They were given a series of psychometric tests which includ-
ed; recognition threshold, sensitivity test and PTC test. Fifteen students were included in the semi trained panel based on their sensitivity and only tasters were selected. In the preliminary session panellists were trained for sensory evaluations; care was taken to avoid physiological errors and bias. They were then trained for threshold study and Quantitative Descriptive Analysis (QDA) (Table 3).

**Psychometric studies**

**Threshold study**
A threshold test for the sugar and intense sweeteners was conducted based on the method given in IS: 5126 (1969) and ASTM 1996. Stock solutions of sugar and intense sweeteners were prepared for the threshold test. From the 1% stock solution of sucrose, a series of dilutions were made representing increasing sweetness concentration. Initially, geometric series was prepared for deciding the concentration for arithmetic series ranging from 0.4 to 2% for sucrose and evaluated by trained panellists. The series for other sweeteners were sucralose (0.001 to 0.005%) and stevia (0.01 to 0.1). These dilutions of sugar and sugar substitutes had a different intensity of sweetness. The panellists were asked to taste the series of solutions arranged in increasing order of concentrations and mark ‘0’ if no stimulus was perceived, ‘?’ if the stimulus was perceived to be different from blank but not recognizable and ‘x’ for recognition threshold value for sweetness.

**Cookie storage studies**
Sugar-free cookies were packed in individual cups made of biodegradable terepoyster and then in 100gms non-leachable pouches comprising of a two-layered laminated material made up of metallized PE and LDPE nylon with GSM of 75mm. The final product was stored at different temperatures and relative humidity to check the stability of the product (Table 4). The stability conditions were decided on the basis of intrinsic and extrinsic factors of the product. The storage studies for diabetic cookies were conducted for three months at ambient temperature that is 27°C and 40°C ±2°C and relative humidity of 75% ±5% respectively. They were analysed on the zero day, third day, seventh day and then fortnightly for 90 days. During this period, the cookies were analysed for physicochemical, microbiological and sensory parameters. (Table 5).

**Macro nutritive composition of cookies**
Macro nutritive composition of cookies included estimation of moisture, total fat, cholesterol, trans fat, protein, total carbohydrate, dietary fibre, calcium, iron, ash, and vitamin A. Moisture content of the cookies (%) was done using the air oven drying method (AOAC: 2005) (105± 1°C for 4 h), fat estimation using soxhlet extraction, protein using Kjeldhal method, total dietary fibre using AOAC 991.43:2005, ash content (AOAC: 2005) and carbohydrates (by difference) content of cookies.
Method for synthesis of biodegradable packaging

Reagent source and analysis

All the reagents, lactic acid (AR), phthalic anhydride, ethylene glycol and stannous chloride, were procured either from E. Merck or Thomas Baker. All the glassware which were used for the laboratory purpose were kept overnight in a 10% (v/v) nitric acid solution and distilled water was used to wash the glassware.

Synthesis of terpolyester

The condensation reaction occurs in two steps. The first step is poly-esterification followed by poly-condensation reaction. Lactic acid, phthalic anhydride and ethylene glycol were taken in proportion of 0.3:1:1 in a three necked round bottom flask. A thermometer was fitted to the neck, a stirrer to the other and Dean-Stark was fitted to third neck as shown in Fig. 2. At first, the temperature was maintained at 140 °C for 7 h and then with constant stirring, the reaction was carried out at 200°C for 10 h and 0.5% stannous chloride was added as a catalyst. The mixture was then poured into a petri dish with a silica gel coating and cooled as shown in Fig. 3. The film is formed polymer is melted in the presence of lactic acid (0.4 mol) phthalic anhydride (1.0 mol) and ethylene glycol (1.0 mol). This is done in three-necked round bottom flask. (Jaemin et al., 2020)

Characterization of terpolyester resin

The synthesized terpolyester so obtained were characterized for acid value, hydroxyl value, no. average molecular weight, UV-Vis Spectroscopy, FT-IR and 1HNMR.

Acid value (ASTM D 1639)

Acid number was determined by dissolving 2.5g polymeric material in chloroform and was titrated against 0.1 N of standardized KOH until endpoint persisted. Phenolphthalein was used as indicator. End point was pink color.

\[
\text{Acid Value} = \frac{5.61 \times V \times N}{M}
\]  

\\(\text{Eq. 1}\\)

Where V= Vol. of KOH, N= Normality of KOH and M= mass of polymer dissolved

Approx. 0.50 g of polymer was taken in 50 ml phthoylating mixture and hydrolysed by adding 100 ml chilled DI water in another flask. Under vigorous stirring, 20 ml benzene was added. The resulting solution was titrated

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**Table 4. Sensory evaluation of diabetic cookies.**

<table>
<thead>
<tr>
<th>Panellists</th>
<th>Code 1 (Control)</th>
<th>Code 2 (Fresh finished product)</th>
<th>Code 3 (after two months of opening the product)</th>
<th>Code 4 (after three months of opening the product)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>5</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>6</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>6</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>7</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>5</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>5</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>6</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>3</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>9</td>
<td>8</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>6</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Average</td>
<td>8</td>
<td>5.2</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>2.4</td>
<td>1.41421356</td>
<td>1.32664992</td>
<td>1.5494</td>
</tr>
</tbody>
</table>

**Table 5. Physico-chemical, microbiological and sensory analysis of cookies.**

<table>
<thead>
<tr>
<th>Method/parameters</th>
<th>Specifications</th>
<th>0 Days</th>
<th>1 Month</th>
<th>2 Months</th>
<th>3 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensory analysis</td>
<td>Crispy texture and flavour</td>
<td>No Rancidity</td>
<td>No Rancidity</td>
<td>No Rancidity</td>
<td>No Rancidity</td>
</tr>
<tr>
<td>Moisture content %</td>
<td>2.08</td>
<td>2.10</td>
<td>2.35</td>
<td>2.50</td>
<td></td>
</tr>
<tr>
<td>Water Activity</td>
<td>0.4785</td>
<td>0.4418</td>
<td>0.5148</td>
<td>0.4086</td>
<td></td>
</tr>
<tr>
<td>Sensory Rancidity</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Total Plate count (10,000/ml)</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>E. Coli (cfu/ml)</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Yeast and Mold (cfu/ml)</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
</tbody>
</table>
against 0.5 N standardized KOH using phenolphthalein as indicator.

\[
\text{Hydroxyl value} = \frac{56.1(V_1 - V_2) \times N}{m} \quad \text{......Eq. 2}
\]

Where \( V_1 \) = vol. of 0.5 KOH used in blank titration, \( V_2 \) = vol. of 0.5 KOH used for polymer solution

The no. average molecular weight was calculated using the following expression:

\[
\text{Mn} = \frac{F \times 10^6}{C} \quad \text{..........Eq 3}
\]

Where \( F \) = functionality of Polymer & \( C \) = Acid value

The UV-Vis spectroscopy of terpolyester was done using ELCO SL 159 and determined the maximum absorption by using solvent chloroform. The maximum absorption of UV of both the terpolyester occur at 205 nm with different intensities. Ratio 0.3:1:1 with intensity 0.981 and ratio 0.4:1:1 with 0.875. The UV-Vis spectroscopy study of terpolyester concluded that there is a transition due to carbonyl chromophore present in the structure. It shows there is a transition in \( n\pi^* \).

**RESULTS AND DISCUSSION**

In present study, the formulated cookies were sugar free, high in soluble fibre \( \beta \)-glucan and had a low glycaemic index (GI). This technology has been developed at lab scale keeping in mind the consumer’s interest in health and special dietetic foods.

**Raw material analysis**
The chemical analysis of the raw material used for the preparation of the cookies is tabulated as under.

**Physical evaluation of cookies**

**Cookie diameter**
Cookies made with non-nutritive sweetener were found to have an average diameter of 4.91 cm. This could be attributed to a lack of sugar content and high fibre content from oats and bran in the cookies. Deshmukh and Bhivagade (2019) reported a decrease in the diameter of biscuits made with 3% sucralose and 0.1% stevia. The decrease in the diameter of the cookies usually occurs during baking due to the production of carbon dioxide by leavening agents and water evaporation. (Faridi et al., 1994). However, cookies made with natural sweetener, i.e. 100% sucrose have been reported to have an average diameter of 5.5 cm (Stone and Sidel, 1998).

**Cookie height**
The cookie height is attributed to gluten development during the baking process. The sugar competes for water over gluten proteins and therefore, the increase in the height of the cookies takes place gradually. Thus, the quantity and kind of sugar used in the formulation directly influence the height of cookies. The mean height of cookies made with non-nutritive sweetener was 2.58 cm as compared to those made with 100% sucrose which was reported to be 1.25 cm. This is because of the lack of hygroscopic nature of non-nutritive sweetener, sucralose that does not compete for water and allows for greater hydration of gluten and heightens the cookies (Handa et al., 2012). Similar observations were made by Deshmukh and Bhivagade (2019) with an increase in the height of biscuits prepared with sucralose and stevia from 4 to 6 mm. Further, the shortening used for sugar-free cookies had nitrogen flushing, which could be responsible for the increased
Sensory acceptability tests
The results of the descriptive analysis of the cookies are shown in Fig. 4. The overall acceptability was found to be 60% by the panellist for cookies with sucralose, having a mean score of 5.7 on a 9 point hedonic scale. The cookies were brown in colour and had pleasant flavour, and sweetness without lingering after the taste of the sweetener. The texture was found to be acceptable but grainy because of the presence of wheat bran and rolled oats. Crumbliness which is a desirable property of cookies, was reported to be 6.33 on a nine-point scale. The visual appearance of the cookies with non-nutritive sweetener was also liked moderately. The overall acceptability of developed formulations was higher in the rating of liking that is between 7-8. The product did not change much with the storage of 90 days at ambient temperature, as depicted in Table 3. However, a consistent decline in the overall acceptability of the product was reported by the judges as compared with the control product after two months and then after three months.

Cookie storage stability studies
Cookie moisture content
The moisture content of the cookies was found to be 2.08 % at 0 month and 2.50 % at the end of the third month. There was no significant increase in moisture content during the storage period of the study and was found in the acceptable limit as given by FSSAI of less than 5%. (Deshmukh and Bhaivagade, 2019) also reported a similar increase in moisture content from an initial 4.4% to 4.69 % at the end of two months of sugar-free multigrain biscuits prepared with sucralose and stevia when stored for two months.

Physico-chemical, microbiological and sensory studies
Stability studies of the product
The accelerated shelf-life studies were carried out for sugar-free cookies after packaging in biodegradable polymer for a period of 90 days, as shown in Table 6. The microbiological studies conducted for yeast, mold, total plate count and coliform revealed that no microbial growth was detected in sugar-free cookies till the end of storage period. Thus, the product is safe to consume.

Macro nutritive composition of cookies
Nutritional evaluation of cookies prepared with non-nutritive sweetener is shown in Table 7. The sugar-free cookies provided a high fibre content of 8.3g per 100 gms with zero trans fats. The cookies had a low glycemic index with high dietary fibre content of 2.5g per serving, out of which β-glucan, a soluble fibre was found to be 0.8g per serving. Thus, offering a healthy alternative for consumers.

Chemical analysis of synthesized terpolyester
The acid value, hydroxyl value and molecular weight of the synthesized terpolyester denotes good barrier properties and optimum mechanical strength as required by any packaging film as shown in Table 8.

(Lindani et al., 2020) reported that the molecular weight of the polymer was lower as compared to the terpolyesters in the present study which was found to be more than 10,000. This signifies the good mechanical strength properties of the polymer.

**Infrared spectroscopy**

Infrared spectroscopy indicated the presence of ester bond and aromatic hydrocarbons. The sharp peak obtained at 1716 cm\(^{-1}\) indicated C=O in aryl ester. The broad peak at 3447 cm\(^{-1}\) indicates presence of O-H structure of alcohol and the presence of two bands at 1134 and 1281 cm\(^{-1}\) indicates the presence of C-O in ester. The bands at 1507 cm\(^{-1}\) and 1575 cm\(^{-1}\) indicate C=C structure in aromatic hydrocarbon, as shown in Fig. 5. The presence of aromatic hydrocarbons implicate a rigid structure of the polymer. This a desirable feature in the production of packaging materials. It has been seen that formation of aliphatic hydrocarbons are responsible for a weak esterification of polymers.

**UV-Vis spectroscopy**

The maximum absorption of UV of both the terpolyesters occurred at 205 nm with different intensities. The ratio of terpolyester A was 0.3:1:1 with absorbance maxima at 0.981 and ratio of terpolyester B was 0.4:1:1 with 0.875. The UV- Vis spectroscopy study indicates that both the terpolymers A and B have transition due to carbonyl chromophore present in the structure. It shows there is a transition in nm\(^{-1}\).

**H-NMR (nuclear Magnetic Resonance Spectroscopy)**

Film formation

The produced terpolyester film was blended with Poly (butylene terephthalate) i.e. PBT with different ratios (Terpolyester: PBT - 6:4, 7:3, and 8:3). Out of which 7:3 ratio blend was selected for further processing. The above blend exhibited good pliability for casting (Fig. 6 & 7).

**Physical testing of packaging film**

**Tensile Strength**

The tensile strength of the developed biodegradable packaging material was performed using tensile strength tester (ASTM). It was found that terpolyester B film had higher percentage (8.7 MPa) of lactic acid, thereby indicating high tensile strength of the polymer (Table 9).

**Durometer hardness test (Shore-A) ASTM D 2240**

The Durometer hardness test was used for measuring the relative hardness of test material. The results indicated that the terpolyester A (LA:EG:PA 0.3:1:1) have shore A - 94 and terpolyester B (0.4:1:1) have A-92.
Both the polymers had optimum hardness indicating good mechanical strength of the packaging material. The standard hardness values are in the range of A: 90-120.

**Thermogravimetric analysis (TGA):**
This test indicates weight loss percentage during processing of the polymer resin. A packaging material that is light in weight is a desirable feature for packaging any commodity. The observed weight loss in terpolyester A resin was 6.9 % at 120 °C, as shown in Fig. 8. This is due to physisorbed water on the resin, which is supported by the presence of three water molecules per unit of resin. In the TGA curve of terpolyester B the weight loss was 4.4 % at 120 °C as in Fig. 9. This is due to physisorbed water on the resin, which supported the presence of only one water molecules per repeating unit of resin.

<table>
<thead>
<tr>
<th>Terpolyester</th>
<th>Ratio of LA:EG:PA</th>
<th>Peak Load (KG)</th>
<th>Break Load (KG)</th>
<th>Tensile Strength(MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.3:1:1</td>
<td>4.85</td>
<td>4.85</td>
<td>7.6</td>
</tr>
<tr>
<td>B</td>
<td>0.4:1:1</td>
<td>5.62</td>
<td>3.44</td>
<td>8.7</td>
</tr>
</tbody>
</table>

**Table 9.** Tensile testing of synthesized terpolyester film.

<table>
<thead>
<tr>
<th>Days</th>
<th>% Weight Loss</th>
<th>Terpolyester A(0.3:1:1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>11 %</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>23.34 %</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>42.67 %</td>
<td>62 %</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Days</th>
<th>% Weight Loss</th>
<th>Terpolyester B(0.4:1:1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>11.31 %</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>27.77 %</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>45.46 %</td>
<td>68 %</td>
</tr>
</tbody>
</table>

**Table 10.** Biodegradation of terpolyester blended film by bacterial species (Bacillus).

Both the polymers had optimum hardness indicating good mechanical strength of the packaging material. The standard hardness values are in the range of A: 90-120.
Test for biodegradability
The synthesized terpolyester A and B blends of polymer films (0.3:1:1 and 0.4:1:1) were tested for their biodegradability by fungal species (Aspergillus niger) and bacterial species (Bacillus). The biodegradation test seen in Tables 10 and 11 was done using bacterial and fungal strains of Bacillus and Aspergillus niger respectively for 60 days. It was observed that the polyester containing higher percentage of lactic acid undergoes greater degradation. The polymer is more susceptible to degradation by fungus than by bacteria. As time elapses, the degradation of polyester increases. The biodegradation also increases discoloration and brittleness in the polymer. It is observed that the biodegradability of Terpolyester B was higher i.e. 75% as compared to terpolyester A which was 68% as shown in Table 10.

Preparation of primary packaging for cookies
The synthesized terpolyester B film was used to manufacture cups, as shown in Fig. 10 and 11, due to its higher tensile strength as well as mechanical strength. This was used as primary packaging for storing the cookies. The processing was done at an elevated temperature of 80°C with the technique of plug-assisted vacuum thermoforming by using Buckner flask as mould and beaker was used as a plug.

Conclusion
The sugar-free cookies with novel biodegradable packaging material were highly acceptable by the consumers and offered a healthy alternative to the diabetic population. It was low in glycemic index and rich in dietary fibre. The RDA for dietary fibre was 30g/2000 kcal per day as per the Dietary Guidelines for Indians, 2020. One serving of these cookies would provide ¼ of the day's requirement. Thus, it is also an exceptionally good source of soluble fibre known for hypo-glycemic and hypo-cholesteremic effects in humans. The developed biodegradable packaging material is one of its kind as it can be used to package food materials without affecting the taste, colour or flavour. It can be moulded in assorted shapes and sizes, providing a lot
of aesthetic value to the commodities without compromising its printability and physical properties. Therefore, diabetic cookies and biodegradable packaging material offers huge potential as a functional food for the food industry after scaling it up

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Conflict of interest

The authors declare that they have no conflict of interest.

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