# Chapter 5

## To develop a functional food product using pectin-iron complex and study its storage stability

## 5.1. Introduction

Fruit leather, also known as fruit roll, is a low-calorie dried pureed fruit sheet that combines fruit pulp, fat or milk solids, and other components. Fruit pulp-based fruit leathers are nutritious and organoleptically acceptable, with high dietary fibre, minerals, vitamins, and antioxidants. It can be molded into desired shapes or sizes (FSSAI, 2011). Fruit leather is a dehydrated confectionary primarily developed from pulpy fruits, including Burdekin plums-apple (Chen et al., 2024), pawpaw-banana (Ohijeagbon et al., 2024), tomato (Basdemir et al., 2024) and apricot (Bagdat et al., 2024). It is a cost-effective and convenient value-added replacement for natural fruits, making them ideal for packing and storage (Raj and Dash, 2022).

Fruit leather is often made by drying at low temperatures for an extended period of time. Fruit leather preserves a high level of phenolic compounds and ascorbic acid. Fruit leathers prepared at moderate temperatures assist in retaining phytochemicals and give the product a pleasing hue (Raj and Dash, 2022). Fruit leathers can be dried using several methods, including sun drying, oven drying, cabinet drying, and dehydrator drying (Sharma et al., 2016).

Pineapple (*Ananas comosus*) is an economically significant perennial herbaceous fruit crop in India. The global annual output of this fruit is around 28 million tons. India is the sixth biggest producer of pineapple, with an annual output of around 1.85 million tons (Ministry of Food Processing Industries, 2023). India's primary pineapple-producing states include West Bengal, Kerala, Karnataka, Tripura, and other portions of the North East region (Yadav et al., 2024). The pineapple market is growing due to its nutritional benefits since it contains carbohydrates, water, crude fibre, protein, micronutrients like calcium, potassium, and manganese, vitamins A, C, and organic acids. Therefore, consuming the same can provide numerous health benefits, including improved digestion and balanced nutrition (Anjaly et al., 2022; Chaudhary and Singh, 2024).

The textural and structural integrity of fruit leather primarily relies on gelling chemicals such as pectin, often used to improve product uniformity and stability. Commercial pectin is frequently utilized in the food sector because it can produce gels in the presence of fruit acids and sugars. However, the growing interest in clearer labelling and useful additives has prompted greater investigation into alternatives to commercial pectin (EFSA ANS

Panel et al., 2017). Pectin complexes, whether obtained from natural sources or modified to increase functioning, are a prospective alternative because of their capacity to improve the texture, nutritional profile, and shelf stability of fruit-based goods. These compounds may have higher gelling characteristics and greater resilience to environmental variables during storage, making them an appealing option for fruit-leather production.

In the present study, mixture design was used for the experimental design and optimization of the formulation of pectin complex-based pineapple leather by measuring the browning index, total phenolic content, and antioxidant activity. Additionally, sensory attributes were examined to determine the product's acceptability. Furthermore, the produced pineapple leather was tested for stability. The pineapple leather was evaluated for water activity, moisture content, browning index, TPC, antioxidant activity, and microbial stability during storage. A statistical study was conducted to compare all of the analyses.

## 5.2. Materials and Methods

### 5.2.1. Preparation of sample

The procured *kew* variety of pineapple (*Ananas comosus*) fruit was disinfected using 5% w/v sodium hypochlorite solution and peeled manually to extract the pulp. The peeled pineapple was ground using a domestic juicer, and the obtained pulp was sieved using a 250-mesh British Standard (BS) sieve for uniform consistency. The PIC (0-2% w/w), sugar (0-20% w/w), potassium metabisulphite (0.2% w/w) was added to the homogenous pineapple pulp according to the I-optimal design and heated up to total soluble solids (TSS) of 78°B. The prepared solution was smeared on butter paper in an aluminium tray and kept for oven drying at 60°C for 12 h. The prepared pineapple leather was cut into 15cm ×9 cm and packed in LDPE pouches for storage.

### 5.2.2. Pectin iron complex (PIC) preparation

Pectin was extracted from Assam lemon peels using the microwave-assisted extraction method, as described in Section 3.2.3.1. Further, extracted pectin was complexed with Fe to produce PIC, as described in section 4.2.3. Pectin (0.5% w/v) was suspended in 1.36 mM iron III chloride hexahydrate ($FeCl_3.6H_2O$) for 180 h at RT. After incubation, the solution was centrifuged at 6000 rpm for 10 min. The PIC was dialyzed against deionized

water for 48 h at RT, freeze-dried for 6 h, and then moved to a sealed container at RT for further developing pineapple leather.

### 5.2.3. Experimental design of the pineapple leather

The data analysis and optimization were conducted using Design Expert 12 (Stat-Easy Co., Minneapolis, MN, USA). The mixture design involves using varying amounts of independent variables to create a blend, with the total of all components being 100% (Sachs, 2012). The mixture design for three independent variables can be expressed as mentioned in Equation 5.1.

$$\sum_{i=1}^{q} xi = x1 + x2 + \ldots + xq = 1 \tag{5.1}$$

Where, $x_I$ denotes the fraction of *i*th component in the mixture.

I-optimal design was used in this investigation to precisely predict over the whole design space. While D-optimal designs are commonly utilized for parameter estimation, the I-optimal design was found to be better appropriate for the goals of this investigation, which centred on reducing prediction error. The I-optimal design yielded 16 experimental runs (including 4 replicates) to optimize the ingredient ratio for pineapple leather.

The independent variables selected for the pineapple leather from the developed PIC were pulp ($x_1$), PIC ($x_2$) and sugar ($x_3$) with ranges 78.05 – 98.92 g, 0 – 2 g and 0 – 20 g, respectively, presented in Table 5.1. These parameters were used to study their effect on the TPC (mg GAE/g extract), DPPH (% RSA), browning index, and sensorial overall acceptance of pineapple leather. A quadratic-designed model was employed to analyse the interaction of ingredients within the designated mixture designs, utilizing regression analysis and ANOVA for statistical evaluation.

The pseudo-components were determined using Equation 5.2.

$$Psx = Cx - \frac{ay}{\Sigma ay} \tag{5.2}$$

In this context, *Psx* denotes the pseudo-component of each constituent, *Cx* represents the actual concentration of the constituent, ay signifies the minimum threshold of the actual

component, and *Σay* indicates the aggregate of the minimum thresholds of the three constituents in the mixture design (Zanela et al., 2015). Table 5.1 presents the actual components and the pseudo-components of each blend.

### 5.2.4. Storage studies

Dehydrated PL samples were packed with 9 mm thick low-density polyethylene (LDPE) at 25°C and were stored at 4°C, 15°C and 25°C for 35 days. PL was analysed every 7 days for water activity ($a_w$), moisture, colour (browning index), pH, total phenolic content, antioxidant activity, hardness, and microbial load during the storage period (Concha-Meyer et al., 2016).

### 5.2.5. Quality attributes of fruit leather

#### 5.2.5.1. Proximate analysis

Proximate analysis such as moisture content, ash content, crude lipid content and crude protein content of PL were determined according to the AOAC method (AOAC, 2010).

#### 5.2.5.2. Extraction method for total phenolic content and Antioxidant activity

The PL extracts for determining total phenolic content and antioxidant activity was slightly modified from Mohd. Esa et al. (2010) method. TPC and antioxidants in PL were extracted using 80% methanol (1:50 w/v) in an incubator shaker for 2 h at RT in the dark. After filtering using Whatman No. 1 filter paper, samples were stored at -20°C for subsequent analysis.

#### 5.2.5.3. Total phenolic content

The total phenolic content (TPC) of PL extracts was determined using the method reported by Raj and Dash (2020), with minor changes. Gallic acid was used as a standard (0–0.1 mg/mL). In a labelled test tube, 0.5 mL of PL extracts, 2.5 mL of Folin-Ciocalteu reagent (10% v/v), and 2.5 mL of 7.5% sodium carbonate solution were added and vortexed. The sample tubes were incubated for 45 min at RT in a dark environment. After incubation, absorbance was measured at 765 nm. The results were represented in milligrams of gallic acid equivalent per gram of extract.

#### 5.2.5.4. DPPH free radical scavenging activity assay

The radical scavenging activity (RSA) of PL extracts for DPPH radical was determined according to Raj and Dash (2020) with minimal adjustments. In a labelled test tube, 0.2 mL of PL extracts, 2 mL of ethanol and 2 mL freshly prepared methanolic DPPH (0.06 mM) were added. After 30 min of incubation in dark conditions at RT, absorbance at 517 nm was measured using a UV Vis spectrophotometer against a blank (ethanol). As a control, equal amounts of solvent and DPPH were used. The RSA was calculated as Equation 5.3.

$$\text{DPPH (\% RSA)} = \left(1 - \frac{Asample}{Acontrol}\right) \times 100 \quad (5.3)$$

Where, Acontrol is the mixture of ethanol and DPPH solution, and Asample is the solution containing PL extract and DPPH solution.

#### 5.2.5.5. Browning index

The findings were presented as Hunter colour indices ($l^*$: brightness, $a^*$: green to red sign, and $b^*$: blue to yellow symbol). The browning index (BI) was computed using Equation 5.4. The value of $x$ in Equation 5.4 was calculated using Equation 5.5 (Nourzad et al., 2024).

$$\text{BI} = \frac{[100(x-0.31)]}{0.17} \quad (5.4)$$

$$x = \frac{a^* + 1.75l^*}{5.645l^* + a^* - 3.012b^*} \quad (5.5)$$

#### 5.2.5.6. Sensory analysis

Acceptability tests of fruit leathers were conducted with 20 semi-trained panellists selected from the university community. The sample was rated based on appearance, taste, texture, and overall sensory score. Ihekoronye and Ngoddy's (1985) 9-point Hedonic scale was employed. The panellists were given a questionnaire with instructions to rate 9 for strongly like and 1 for extremely dislike (Offia-Olua and Ekwunife, 2015).

#### 5.2.5.7. pH

pH was measured by dissolving 1 g of respective PL in 50 mL of distilled water. After 5 min incubation, pH was measured by using a pH meter.

#### 5.2.5.8. Water activity

Water activity ($a_w$) was measured using an AquaLab Pawkit water activity meter (Meter group, Court Pullman, WA, USA) at 21 ± 2°C (AOAC, 2010).

#### 5.2.5.9. Microbial analysis

Microbial analysis was performed for fresh as well as PL samples stored during the storage period of PL. To prepare, 2 g of PL was sliced into smaller pieces, vortexed, and agitated in 18 mL of 0.1% peptone solution in a test tube. Serial dilutions up to $10^{-2}$ were prepared in duplicate. The standard plate count was performed using the pour plate technique with 1 mL sample solution and 15 mL nutrient agar, incubated at 35°C for 48 h (AOAC 966.23) (AOAC, 2000). The yeast and mold count were performed using the spread plate method (AOAC 997.02) with a 0.1 mL sample solution on a potato dextrose agar plate. The plate was incubated at 25°C for 72 h. Colony counts were expressed as the natural logarithm of colony-forming units per gram of sample (log CFU/g) (Chen et al., 2024).

### 5.2.6. Statistical analysis

Data analysis was performed using the one-way analysis of variance (ANOVA), and Duncan's mean comparison test was conducted at a significance level of $p = 0.05$ to identify any variations among treatments. The experiment is conducted in triplicates, and data is performed in mean ± standard deviation.

## 5.3. Results and Discussion

### 5.3.1. Experimental modelling of mixture design and optimization

The PL parameters were chosen in accordance with the mixture design to create a sensory acceptable and homogeneous texture based on pineapple pulp, PIC, and sugar. The preliminary testing revealed that the PL was unable to produce the appropriate texture leather without sugar and PIC owing to a lack of binding agent. The constraints were

solved by integrating PIC and sugar via mixture design and a sodium metabisulphite as preservative (0.5% w/w). Table 5.1 shows the preparation of suspension for each constraint, as well as the pseudo constraints and findings.

The model adequacy can be analysed through the statistical parameter correlation coefficient ($R^2$) and adjusted $R^2$. The $R^2$ and adjusted $R^2$ values for each dependent variable did not differ significantly, and the non-significant terms were neglected from the model as seen in Table 5.2. Response surface plots along with the contours of PL for TPC, DPPH antioxidant activity, BI, and total sensory score are displayed in Figure 5.1. The L-pseudo components of the equations for various PL quality attributes obtained using ANOVA are the mixed component coding, sometimes referred to as L-pseudo equations. The empirical models derived from fitting the answers to the mixed design are represented by these equations. The response and process variables generated by the mathematical model are displayed in Table 5.3.

In order to determine the best PL formulation, a mixture design was used to examine the effects of pineapple pulp, PIC, and sugar on TPC, antioxidant activity, browning index, and overall sensory acceptance. The idealconditions for the formulation of PL were determined to be pineapple pulp (90.588%), PIC (1.833%), and sugar (7.579%), on the basis of impact of independent factors on the response values for different PL attributes. To confirm these anticipated conditions, the formulation was evaluated in duplicate tests; the findings, as indicated in Table 5.4, were found to be comparable to the predicted values. The outcomes demonstrate that an effective PL can be created using this formulation.

**Table 5.1** Responses of the independent variables for the pineapple leather prepared with varying concentrations of pineapple pulp, PIC and sugar.

| Runs | Independent variables | | | | | | Dependent variables | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | In real concentration (%) | | | In-Pseudo components | | | Total Phenolic content (mg GAE/g extract) | DPPH (% RSA) | Browning index (AU) | Overall sensory score |
| | Pineapple pulp ($x_1$) | PIC ($x_2$) | Sugar ($x_3$) | Pineapple pulp ($x_1$) | PIC ($x_2$) | Sugar ($x_3$) | | | | |
| 1 | 89.28 | 2 | 8.72 | 0.487 | 0.000 | 0.513 | $157.36\pm2.20^{de}$ | $85.01\pm1.66^{b}$ | $0.018\pm0.001^{d}$ | $8.0\pm0.52^{a}$ |
| 2 | 87.13 | 1.08 | 11.79 | 0.585 | 0.042 | 0.373 | $148.15\pm9.49^{e}$ | $83.45\pm4.36^{c}$ | $0.024\pm0.002^{c}$ | $7.8\pm0.45^{a}$ |
| 3 | 84.52 | 2 | 13.48 | 0.704 | 0.000 | 0.296 | $142.95\pm1.99^{f}$ | $83.57\pm2.07^{c}$ | $0.017\pm0.001^{d}$ | $7.4\pm0.99^{b}$ |
| 4 | 95.05 | 0 | 4.95 | 0.225 | 0.091 | 0.684 | $197.24\pm7.24^{c}$ | $83.69\pm0.20^{c}$ | $0.02\pm0.002^{d}$ | $7.5\pm0.29^{b}$ |
| 5 | 92.21 | 0.33 | 7.46 | 0.354 | 0.076 | 0.570 | $216.28\pm16.56^{b}$ | $83.93\pm0.83^{c}$ | $0.011\pm0.001^{f}$ | $7.7\pm0.21^{a}$ |
| 6 | 87.13 | 1.08 | 11.79 | 0.585 | 0.042 | 0.373 | $145.28\pm6.16^{e}$ | $80.34\pm0.20^{f}$ | $0.022\pm0.002^{c}$ | $7.7\pm0.19^{a}$ |
| 7 | 83.67 | 0 | 16.33 | 0.742 | 0.091 | 0.167 | $150.07\pm2.54^{e}$ | $84.05\pm1.20^{c}$ | $0.009\pm0.003^{f}$ | $7.3\pm0.91^{c}$ |
| 8 | 95.05 | 0 | 4.95 | 0.225 | 0.091 | 0.684 | $196.34\pm12.23^{c}$ | $82.78\pm0.21^{d}$ | $0.019\pm0.001^{d}$ | $7.3\pm0.03^{c}$ |
| 9 | 93.49 | 2 | 4.51 | 0.296 | 0.000 | 0.704 | $229\pm11.32^{a}$ | $85.13\pm0.21^{b}$ | $0.026\pm0.002^{b}$ | $7.5\pm0.08^{b}$ |
| 10 | 98.92 | 1.08 | 0 | 0.049 | 0.042 | 0.909 | $166.84\pm13.23^{d}$ | $83.05\pm0.82^{c}$ | $0.011\pm0.001^{e}$ | $7.0\pm0.14^{d}$ |
| 11 | 98.92 | 1.08 | 0 | 0.049 | 0.042 | 0.909 | $167.34\pm9.46^{d}$ | $82.61\pm0.31^{d}$ | $0.013\pm0.002^{e}$ | $6.9\pm0.19^{d}$ |
| 12 | 80.84 | 2 | 17.16 | 0.871 | 0.000 | 0.129 | $136.85\pm1.17^{g}$ | $75.06\pm1.15^{g}$ | $0.006\pm0.002^{g}$ | $7.3\pm0.20^{c}$ |
| 13 | 78.05 | 1.95 | 20 | 0.998 | 0.002 | 0.000 | $83.51\pm9.00^{h}$ | $77.39\pm2.35^{g}$ | $0.006\pm0.001^{g}$ | $8.1\pm0.51^{a}$ |
| 14 | 87.13 | 1.08 | 11.79 | 0.585 | 0.042 | 0.373 | $151.56\pm8.53^{e}$ | $86.33\pm0.63^{a}$ | $0.026\pm0.001^{b}$ | $7.8\pm0.32^{a}$ |
| 15 | 87.13 | 1.08 | 11.79 | 0.585 | 0.042 | 0.373 | $148.63\pm6.42^{e}$ | $83.73\pm0.37^{c}$ | $0.027\pm0.001^{b}$ | $7.7\pm0.17^{a}$ |
| 16 | 79.87 | 0.13 | 20 | 0.915 | 0.085 | 0.000 | $161.65\pm9.00^{d}$ | $82.25\pm0.21^{e}$ | $0.029\pm0.001^{a}$ | $7.7\pm0.19^{a}$ |

*Different superscript lower case letters show differences between the rows (runs) *($p<0.05$)*

**Table 5.2.** The ANOVA test results indicating the effect of independent variables on dependent variables for pineapple leather

| | **Total Phenolic content (mg GAE/g extract)** | **DPPH (% RSA)** | **Browning index (AU)** | **Overall sensory score** |
|---|---|---|---|---|
| **Model** | Cubic | Quadratic | Cubic | Special quartic |
| **Model (*F* value)** | 268.61 | 4.53 | 17.58 | 38.24 |
| **Model (*p* value)** | <0.0001 | 0.0204 | 0.0012 | <0.0001 |
| **Lack of fit (*F* value)** | 5.93 | 1.04 | 4.31 | 1.05 |
| **Lack of fit (*p* value)** | 0.0590 | 0.4834 | 0.0926 | 0.4164 |
| ***$R^2$*** | 0.9975 | 0.6936 | 0.9635 | 0.9776 |
| **Adjusted *$R^2$*** | 0.9938 | 0.5404 | 0.9087 | 0.9521 |
| **C.V. (%)** | 1.68 | 2.35 | 13.03 | 0.97 |

**Table 5.3** Predicted model equations indicating effect of each mixture component and their interactions on dependent variables of pineapple leather

| **S.No.** | **Response** | **Equation in terms of pseudo components** |
|---|---|---|
| 1 | Total Phenolic content (mg GAE/g extract) | $Y = +360.23x_1 + 2.293E+06x_2 - 21.19x_3 - 3.553E+06x_1x_2 - 3.644E+06x_2x_3 + 2.629E+06x_1x_2x_3 + 1.263E+06\ x_1x_2(x_1-x_2) - 799.38x_1x_3(x_1-x_3) - 1.373E+06x_2x_3(x_2-x_3)$ |
| 2 | DPPH (% RSA) | $Y = +80.09x_1 + 249.11x_2 + 81.53x_3 + 15.69x_1x_3$ |
| 3 | Browning index (AU) | $Y = +0.0571x_1+50.44x_2+0.0223x_3- 0.1420x_1x_3$ |
| 4 | Overall sensory score | $Y = +7.66x_1 – 521.68x_2 + 7.58x_3 + 541.45x_1x_2 + 587.57x_2x_3 + 2063.9x_1x_2^2x_3 - 265.73x_1x_2x_3^2$ |

[a] $x_1$, $x_2$ and $x_3$ were the mixture components, pineapple pulp, PIC and sugar, respectively.
[b]By applying BER "backward elimination regression" procedure, non-significant interactions were removed from the equations. Only the variables significant at $p<0.01$, $p<0.05$ and $p<0.1$ levels were selected for the predicted model construction.

### 5.3.2. Effect of dependent variables on properties of PL

The effect of dependent variables such as total phenolic content, DPPH free radical scavenging activity, browning index, and overall sensory score on properties of PL has been discussed in further sections.

#### 5.3.2.1. Total phenolic content of PL

TPC variated in between the range of 83.51-229 mg GAE/g extract (Table 5.1). The TPC was seen to decrease with the increase in the sugar content, as seen in Figure 5.1a, due to a lower relative concentration of phenolics sourced from pineapple pulp and PIC. It can be seen that TPC of PL was much higher than those of other fruit leathers such as chokeberry fruit leather (20.43 ± 0.59 mg GAE/g DM) (Olcay et al., 2024), pawpaw-banana fruit leather (0.61 to 0.86 mg/GAE g) (Ohijeagbon et al., 2024), Burdekin plums-apple fruit leather (6.6-18.9 mg GAE/g DW) (Chen et al., 2024), and persimmon leather (29.91-37.46 mg/g) (Mohamed et al., 2018). The differences in TPC of fruit leathers could be due to difference in that of plants.

**Table 5.4** Experimental and RSM predicted dependent variable values at optimum conditions for PL

| S.No | Dependent variables | Experimental values | Predicted values | Relative deviation (%) |
|---|---|---|---|---|
| 1 | Total phenolic content (mg GAE/g extract) | 88.36±2.80 | 83.51 | 5.48 |
| 2 | DPPH (% RSA) | 81.01±1.43 | 84.74 | 4.59 |
| 3 | Browning index | 0.017±0.001 | 0.018 | 5.88 |
| 4 | Overall sensory score | 8.2±0.14 | 8.09 | 1.32 |

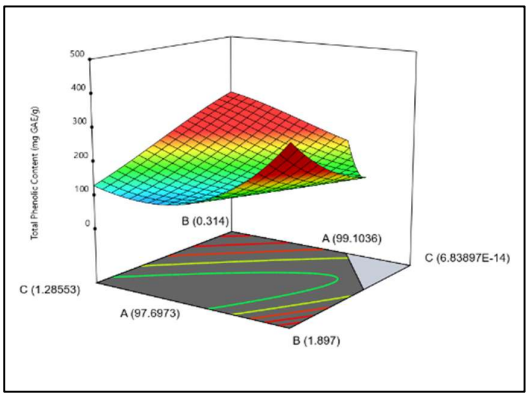
Results are expressed as Mean ± Standard deviation

#### 5.3.2.2. DPPH free radical scavenging activity of PL

The Table 5.1 shows the results varied between the range of 75.06-86.33% RSA. The DPPH % RSA activity revealed an increase corresponding to the elevated concentrations of pineapple pulp and PIC, similar to the findings of TPC (Figure 5.1c and 5.1d). This could be due to the relationship between TPC and antioxidant activity, indicating that extracts with high TPC had strong radical scavenging efficacy (Osman et al., 2020). It can be seen that the DPPH (% RSA) of PL was slightly lower than chokeberry fruit leather (93.89% RSA) (Olcay et al., 2024) while it is similar to that of persimmon leather (83.53-87.08%) (Mohamed et al., 2018).

### 5.3.2.3. Browning index

The browning index was derived using colour coordinates ($L^* a^* b^*$) to assess the change in visual quality. BI helps to determine the degree of brown colour that formed following dehydration. The values of the browning index range from 0.029 to 0.06, as shown in Table 5.1. The cubic model was the most appropriate statistical model for the values of the

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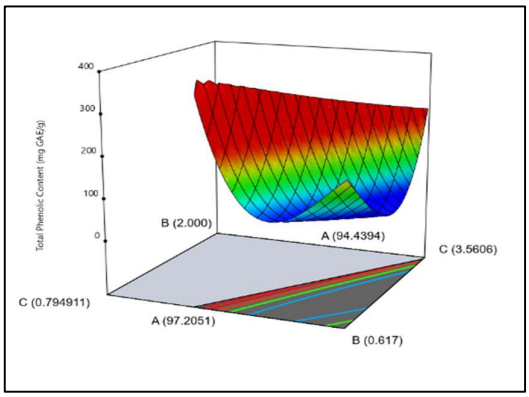


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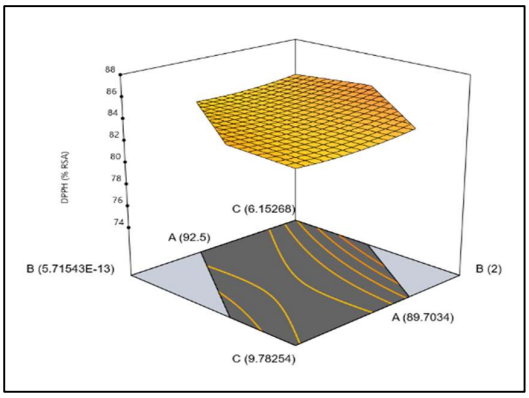


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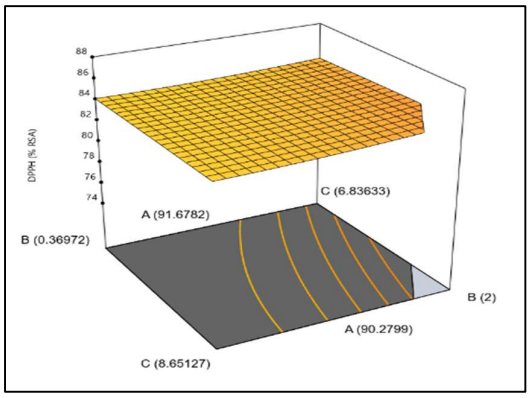


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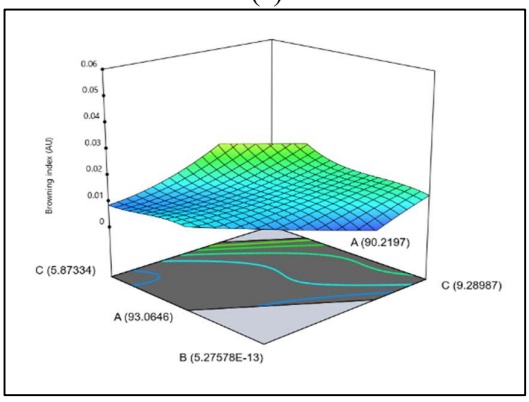


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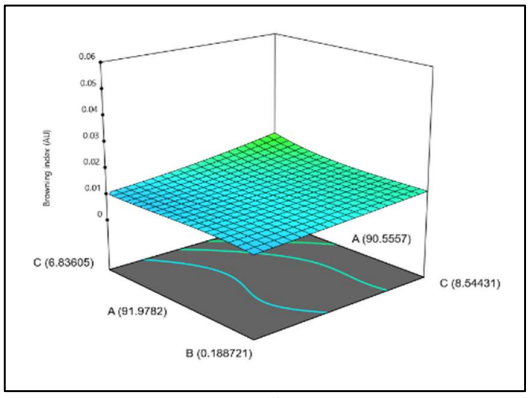


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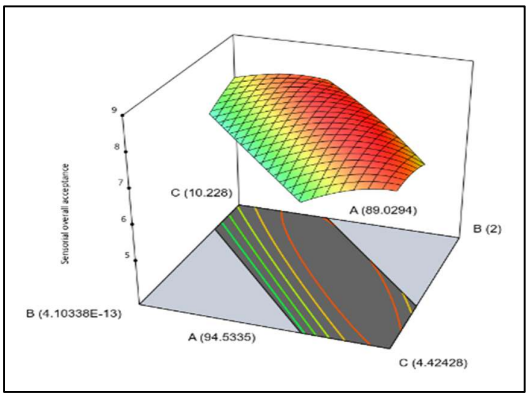


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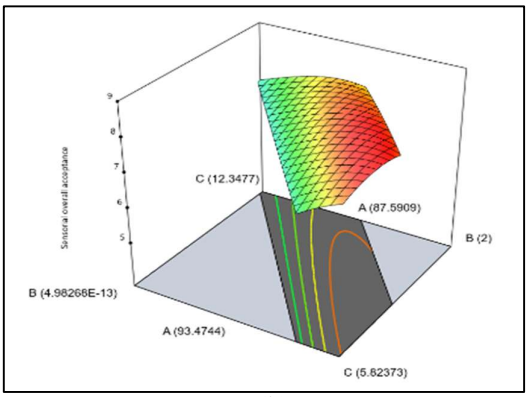



(h)

**Figure 5.1**. RSM plots depicting effects of interaction between pineapple pulp, PIC and sugar on TPC (a,b), DPPH antioxidant activity (c,d), BI (e,f) and overall sensory acceptance (g,h) in PL

browning index. It can also be clearly seen from Table 5.3 and Figures 5.1e and 5.1f that the interaction of pineapple pulp and sugar is negatively influencing BI values. The browning index for persimmon leathers and kiwi leathers ranged from 0.1-0.139 and 11.03-15.34, respectively (Mohammed et al., 2018; Barman et al., 2021). The reduced BI in the present study could be due to the fruit characteristics, the formulation's contents, especially the nature of hydrocolloids, the drying conditions, and the spreading thickness (Tontul and Topez, 2018).

### 5.3.2.4. Overall sensorial acceptance

The sensory score of PL leather is mentioned in Table 5.1. The results show that with the increase in the concentration of pineapple pulp and sugar caused a significant increase in the average sensory score of PL. The PL with the highest sensory score (8.1 ± 0.51) was found to have 1.95% PIC, 20% sugar, and 78.05% pulp (Figure 5.1g and 5.1h). The special quartic model was the most appropriate statistical model for the values of the overall sensory acceptance as the model is significant and lack of fit is non-significant for the same. The higher the percentage of PC in the pineapple pulp content in the leather, the higher the browning index of PL. Leathers with higher concentrations of PIC were less desirable.

Additionally, it was observed that the fruit leather made with 20% sugar had the greatest overall sensory score, at around 8.1 ± 0.51. With a mean score of 6.9 ± 0.19 for the overall sensory score, PL with no sugar had the lowest score. Therefore, it is evident that the sugar-prepared PL exhibits a higher sensory score for texture, flavour, and colour than the sugar-free variant.

**Table 5.5.** Proximate composition (on wet basis) and iron content of formulated PL

| S.No | Parameters | Results (g/100 g) |
|---|---|---|
| 1 | Moisture content | 9.75±0.10 |
| 2 | Carbohydrates | 81.94±0.09 |
| 3 | Crude fat | 5.43±0.05 |
| 4 | Crude fibre | 1.90±0.17 |
| 5 | Crude protein | 1.50±0.20 |
| 6 | Total ash content | 1.38±0.05 |
| 7 | Iron content | 0.0048±0.01 |

*Results are expressed in Mean ± Standard deviation

### 5.3.3. Proximate composition of formulated PL

The proximate composition of PL is mentioned in Table 5.5. Since fruit leathers are naturally low in fat and high in fibre and carbohydrates, they are typically seen as a healthy snack. The ash content of PL was higher than that of *lapsi* fruit leathers (0.65-1.09%) (Yadav et al., 2022), which might be due to the pectin complex as one of the ingredients.

### 5.3.4. Quality attributes of PL during the storage period of PL

During storage period of PL, various quality attributes such as moisture, pH, water activity, browning index, total phenolic content and antioxidant activity was analysed for PL at regular intervals of 7 days.

#### 5.3.4.1. Moisture, water activity and pH

Figures 5.2a and 5.2b show PL's moisture content and water activity over the storage period of 35 days. At day 0, the moisture content in PL was 13.99 ± 1.31% wb. The moisture content in PL increased with time during storage at 4°C, 15°C and 25°C. At 4°C,

there was no significant difference *($p<0.05$)* in the moisture content of PL until 14 days; however, then there was a 20% increase in moisture content from 14 days to 21 days of PL. A similar trend was seen when PL was stored at 15°C and 25°C, however, the highest moisture content (22.75 ± 1.92% wb) was observed at 35 days when PL was stored at 25°C. This could potentially be attributed to the packaging material, specifically LDPE, which exhibits partial permeability, allowing moisture to be absorbed from the surrounding environment (Sagar, 2015). Similar results were reported for *aonla* leather by Sagar (2015), where moisture content was increased with an increase in storage temperature and storage days. On the contrary, Irwandi et al. (1998) reported that despite durian leather showing the highest changes in moisture content and water activity in LDPE packaging, moisture content decreased with increased storage time.

The water activity variations in PL were comparable to the changes in moisture content (Figure 5.2b). Water activity and moisture content were clearly correlated; the higher the moisture level, the higher the water activity. The PL's low water activity (0.6151) contributed to its shelf life since most microbial activity happens at values higher than 0.7 (Ruiz et al., 2012). The water activity for PL stored at 25°C exhibited relatively lower values than that of 4 and 15°C. The values of water activity of PL stored at 25°C are similar to the snake fruit leather (0.64-0.69) (Purwandari et al., 2018), apple leather (0.70-0.71) (Ruiz et al., 2012), but higher than that of apple and quince leather (0.56-0.69) (Torres et al., 2015) and apple-blackcurrant leather (0.50) (Diamante et al., 2013). This might be due to the water in the product bonds to the matrix more efficiently. In contrast to colder storage, samples stored at 25°C stabilise bound water, reducing water activity. Moreover, higher water activity (0.65-0.70) could promote browning and make the product prone to spoilage by osmophilic yeasts and xerophilic fungi, limiting storage stability (Purwandari et al., 2018).

The pH ranged from 3.73 to 3.91 for PL stored at different temperatures for 35 days (Figure 5.2c). There was no significant difference. Azeredo et al. (2006) reported similar results for mango leather (pH 3.5-3.8) over a period of 6 months at 25°C.

#### 5.3.4.2. Browning index

The Browning Index (BI) of PL maintained at 4°C, 15°C, and 25°C for 35 days is shown in Figure 5.2d. The BI generally rises with storage time at all temperatures, signifying a series of browning processes in the fruit leather. Similar results were seen in apple leather when stored for 7 months (Ruiz et al., 2012). The Maillard reaction and enzymatic browning resulting in increased BI are often at higher temperatures. However, PL samples held at 4°C show a greater BI than those stored at 15°C and 25°C throughout the storage period. This might be due to pineapple having a high concentration of reducing sugars, which speeds up the Maillard reaction (Nayaka et al., 2022) and permits browning to occur even at lower temperatures. Although pineapple is a good source of vitamin C, as it decomposes over time, it releases substances that contribute to browning mechanisms, which can lead to oxidative browning (Giacalone et al., 2019). Another reason could be due to some temperature-sensitive enzymes in pineapple, including polyphenol oxidase (PPO), which may continue to function at lower temperatures and intensify browning reactions (Liu et al., 2024). Moreover, Maillard browning significantly increased in PL as water activity increased (Ruiz et al., 2012), variations in moisture content linked to each temperature may also influence the severity of browning (Huang and Hsieh, 2005). While browning increases with storage duration at all temperatures, the highest rates occur consistently at 4°C, suggesting complex temperature-related interactions in pineapple fruit leather.

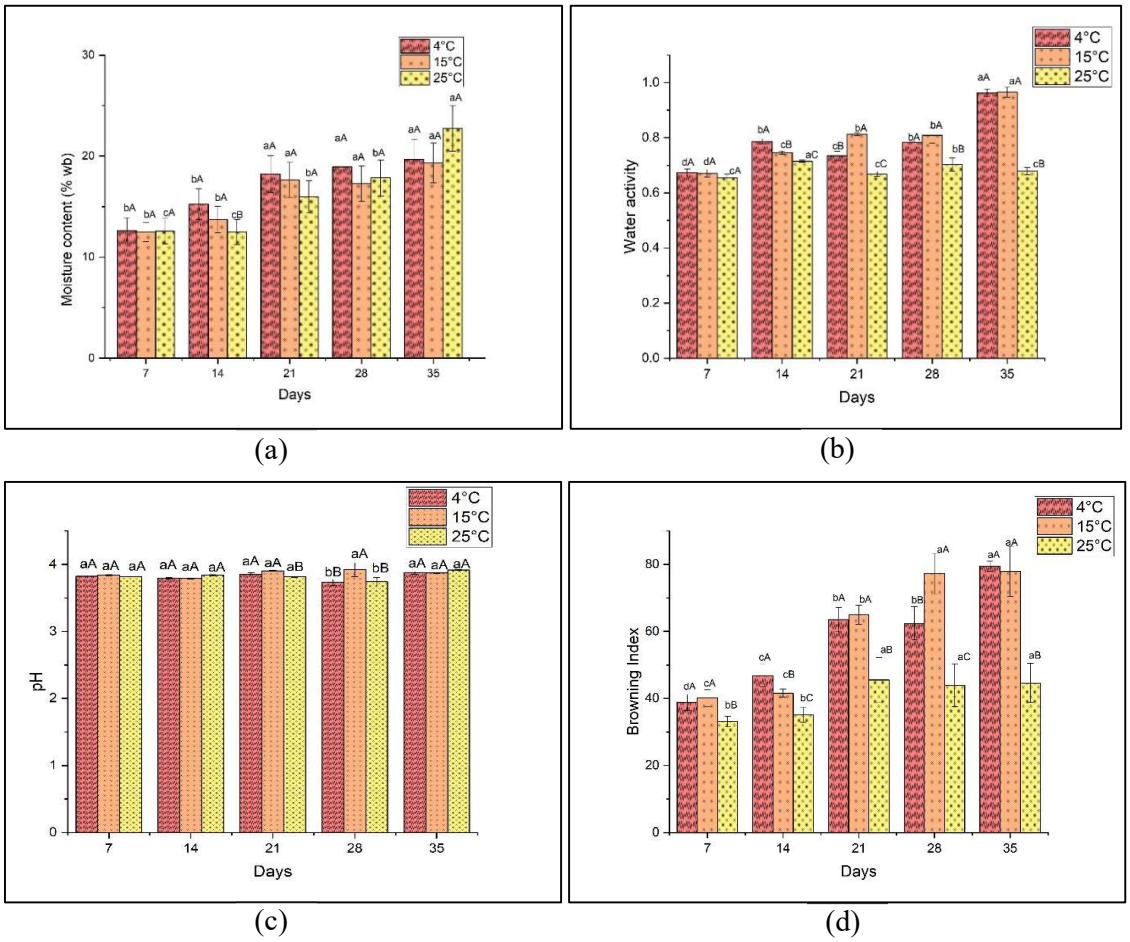
#### 5.3.4.3. Total phenolic content and antioxidant activity

The TPC and DPPH antioxidant activity of PL was seen to be decreasing with an increase in storage duration and storage temperature. At 4 and 15°C, there was no significant difference in the values of TPC of PL until 14 days, however, there was around an 8-14% decrease in the TPC values until the 21$^{st}$ day. Meanwhile, TPC exhibited a significant reduction in the TPC values from the 7th day itself when PL was stored at 25°C (Figure 5.2e). In contrast, when PL were held at 4 and 15°C, their DPPH scavenging activity was considerably reduced compared to day 7 itself. The DPPH activity in PL was found to be 69.44% (Figure 5.2f), which is slightly more than the 64.33% recorded in pineapple fruit leather treated by extrusion cooking (Sharma et al., 2016).

Moreover, TPC and DPPH of PL stored at 4°C and 25°C showed strong positive correlation values of 0.90 and 0.82, respectively, while PL stored at 15°C exhibited a comparatively weak but positive correlation coefficient of 0.64. This positive relation between TPC and DPPH could be due to the possibility that both methods rely on the mechanism of tendency to donate hydrogen (Katsube et al., 2004).

### 5.3.4.4. Texture analysis

Variations in the hardness of PL during the storage period is represented in Figure 5.2g. It can be seen clearly that PL stored at 25°C exhibits comparatively higher hardness than that stored at 4°C and 15°C. Moreover, the hardness of PL stored at all temperatures significantly reduced in the 3rd week compared to the first two weeks, indicating that PL became slightly softer with an increase in storage time. This could be due to increased moisture content (Figure 5.2a) in PL noted on day 21, resulting in decreased intermolecular forces contributing to a decrease in hardness. In addition, this could be enhanced due to internal moisture redistribution and structural relaxation that happened with storage time.



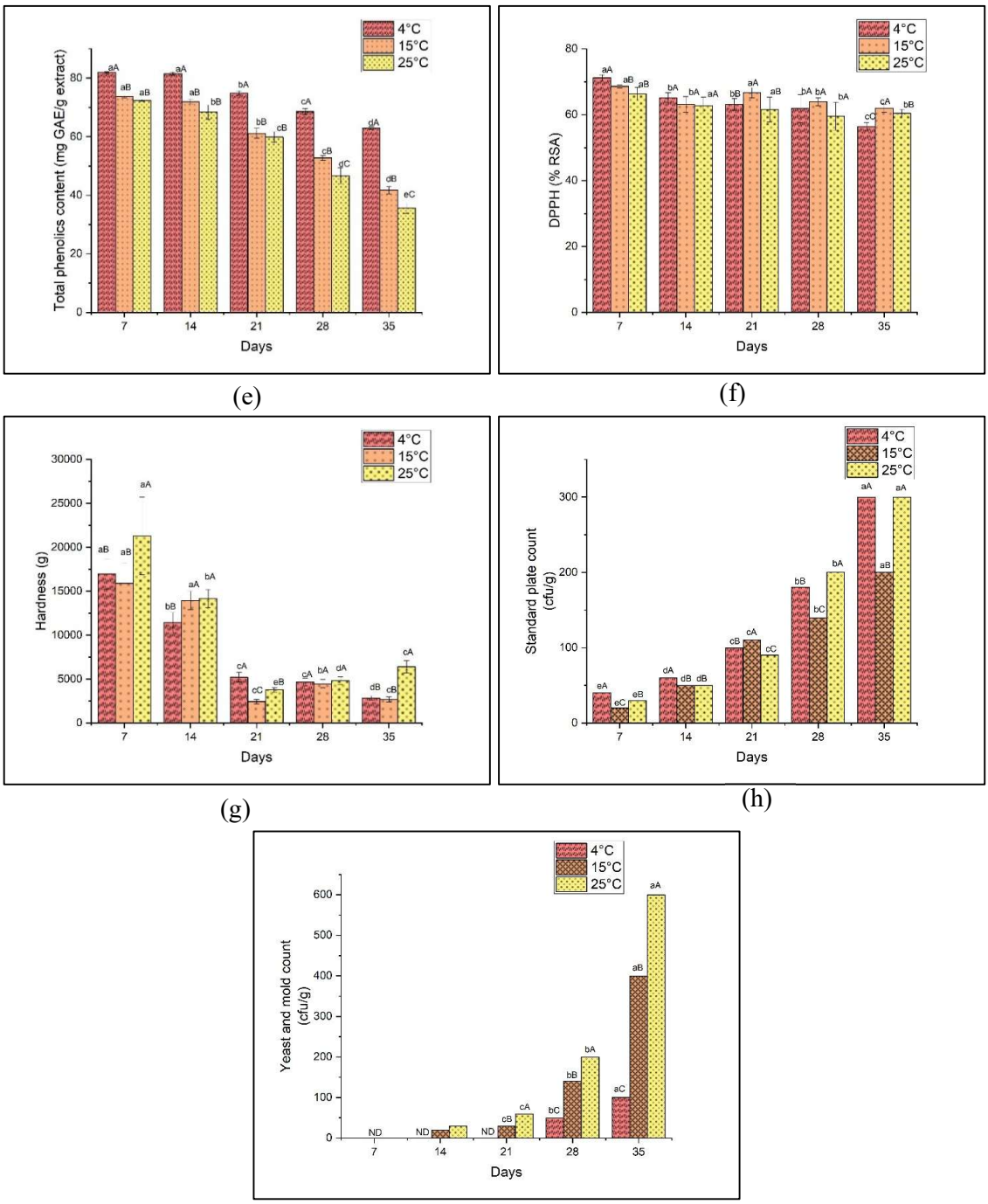



**Figure 5.2.** Effect of storage temperature and time on (a) moisture content, (b) water activity, (c) pH, (d) BI, (e) TPC, (f) DPPH antioxidant activity, (g) hardness, (h) standard plate count and (i) yeast and mold count of PL. Different uppercase letters and different lowercase letters in the graphs differ significantly between bars (temperature) and between clusters (time) at $p<0.05$ in Duncan's multiple comparison posthoc tests.

*ND refers to Not detected

### 5.3.4.5. Microbiological quality

Fruit leathers are classified as intermediate moisture foods (IMFs) because of their moderate water activity (0.60-0.85), which inhibits most bacterial development while allowing slow growth of osmophilic yeasts and xerophilic moulds (Rahman, 2020). Fruit leather's high sugar content is one of the primary causes of spoilage; while it acts as a natural preservative by lowering water activity, it also creates conditions favourable for spoilage organisms such as *Saccharomyces rouxii*, *Aspergillus echinulatus*, and *Monascus bisporus*, which thrive in osmotic and acidic environments (Jay et al., 2005).

The standard plate count for PL stored at 4°C, 15°C and 25°C were in the range of 40-300 CFU/g, 20-200 CFU/g and 30-300 CFU/g, respectively (Figure 5.2h). In addition, the range of yeast and mold count was 0-100 CFU/g, 0-400 CFU/g and 0-600 CFU/g for PL stored at 4°C, 15°C and 25°C, respectively (Figure 5.2i). Similarly, Ezekiel and Olukuewu (2012) found low total mould counts in mango-carrot leather after 60 days of storage, ranging from undetectable to $1.3\times10^2$ CFU/g at 4°C and $1.5\times10^2$ CFU/g at 28°C. In contrast, pawpaw-banana leather exhibited a higher total bacterial count, ranging from $0.5\times10^3$ to $10.0\times10^3$ CFU/g (Ohijeagbon et al., 2024).

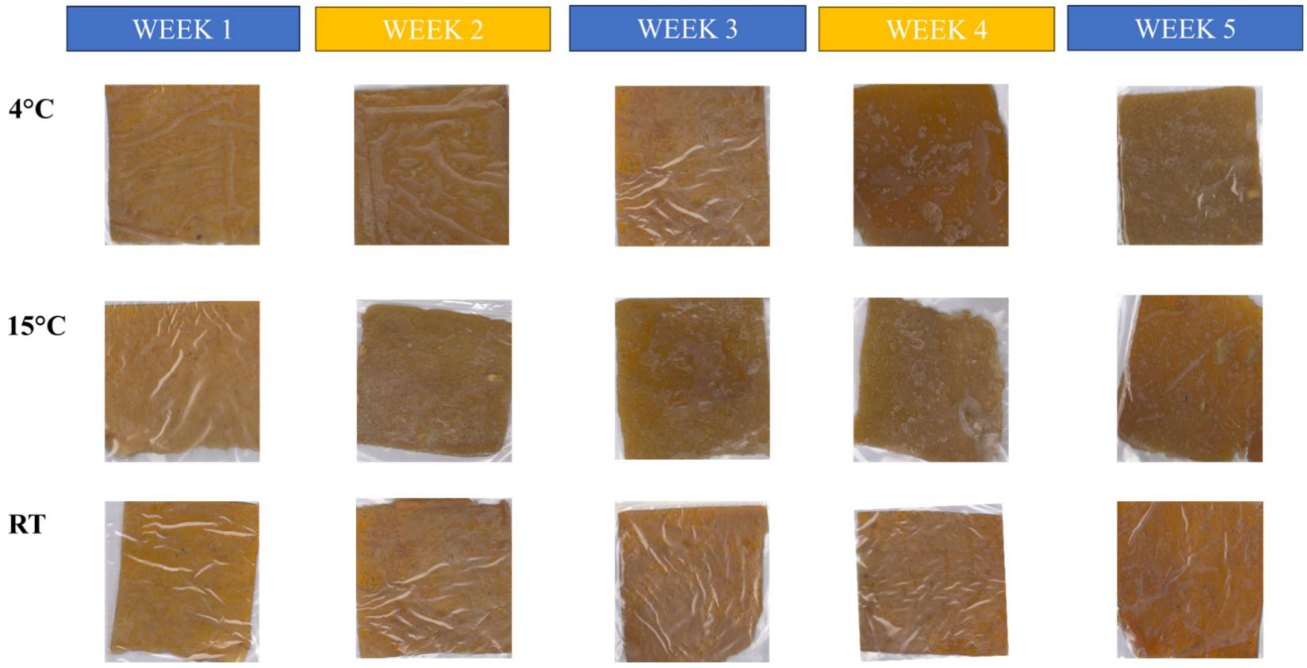



**Figure 5.3**. Images of pineapple leather during storage studies

According to the FSSAI (2018), the aerobic plate count and total mold and yeast should be less than $4\times10^4$ CFU/g and $1\times10^5$ CFU/g, respectively for dehydrated fruits and vegetables. The results indicate that the PL stored at all temperatures after 35 days were safe for consumption as the microbial load was less than 4 log CFU/g. This might be due to the low pH and water activity, as these are unfavourable conditions for microbial growth and survival in PL (Safaei et al., 2019).