DOI: http://doi.org/10.4038/tar.v30i2.8304 **Tropical Agricultural Research Vol. 30 (2): 1 – 12 (2019)**

# Effect of pH, Temperature and Time Combinations on Yield and Degree of Esterification of Mango Peel Pectin: A Box-Behnken Design Based Statistical Modelling

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***ABSTRACT:*** *A 3-factor-3-level Box-Behnken design was employed to determine the effect of conditions: pH, temperature and time on yield and degree of esterification (DE) of mango peel pectin obtained using acid extraction method. Fifteen experimental runs with different combinations of pH (1.3, 2.5 and 3.7), temperature (60, 75 and 90 $^{o}$C) and time (45, 90 and 135 min) were performed on mango peel collected from fruit processing industry. Acid extraction method was used to extract pectin. Yield and the DE of mango peel pectin varied from 6.1 to 16.3% (dry weight basis) and 45.5 to 87.5%, respectively. Interactive effects of pH, temperature and time on the DE were significant at P<0.050. The empirical quadratic second degree polynomial model developed for DE in the study was significant (P=0.000) and well fitted to all experimental data with $R^2$ of 99.48. The study highlighted that mango peel from fruit processing industry can be used to produce high methoxyl (DE > 50 %) or low methoxyl pectin (DE < 50 %) by controlling conditions during extraction and the model could be used to predict the DE of mango peel pectin for given conditions.*

***Keywords****: Box-Behnken design, Degree of esterification, Fruit industry waste, High and low methoxyl pectins, Mango peel*

## INTRODUCTION

Pectin is a polysaccharide of galacturonic acids with branches of neutral sugars such as L-rhamnose, L-arabinose and D-galactose (Wai *et al*., 2010). Galacturonic acids in pectin are methyl esterified to various extents (Wang *et al*., 2017). The ratio of esterified galacturonic acid units to total galacturonic acids determines the degree of esterification (DE) (Flutto, 2003). Based on the DE, pectin is classified as "High methoxyl (HM) pectin" (DE > 50 %) and "Low methoxyl (LM) pectin" (DE < 50 %) (Ranganna, 1986; Thakur *et al*., 1997). The DE determines gelling properties, solubility, emulsion activities, emulsion stability and release effects of pectin in complex food matrices, thus plays a unique role in food manufacturing (Srivastava and Malviya, 2011; Müller-Maatsch *et al*., 2016). However, as pectin is a constituent in plant cell wall, its yield and the DE depend on the chemical composition of plant cells and structure of pectin. Furthermore, nature of raw material,

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method and conditions such as pH, temperature, time, ionic strength of solution, solid: solution ratio of extraction affect yield and the DE of pectin (Yeoh *et al*., 2008; Begum *et al*., 2017; Sandarani, 2017).

Mango fruit is composed of approximately 11 – 18% peel, 14 – 22% seed and the rest being flesh (Mitra *et al.,* 2013). In Sri Lankan context, mango peel and seed account for 51% of total fruit waste discarded by the fruit processing industry (Wathsala *et al*., 2017). As mango peel contains nearly 12 - 18.5% pectin (Koubala *et al*., 2008; Girma and Worku, 2016), investigation of mango peel for pectin extraction is useful. Different extraction conditions are used to obtain pectin from fruit peels (Begum *et al*., 2017; Sandarani, 2017). Therefore, it is important to identify appropriate extraction conditions to obtain maximum possible yield of pectin. Hence, this study was conducted to investigate the effect of extraction conditions namely pH, temperature and time on yield and the DE of mango peel pectin and to optimize these conditions to extract maximum possible pectin by employing Response Surface Methodology (RSM).

## METHODOLOGY

### Collection and preparation of materials

Fresh mango peel was obtained from fruit processing plants at CBL Natural Foods (Pvt) Limited, Minuwangoda, Sri Lanka and Kist Processing Plant, Kilinochchi, Sri Lanka. Peels were transported to the laboratories within 2 - 3 h, cleaned, sorted, washed twice in potable running water and left for 10 min for moisture draining. The peels were disintegrated into pieces of approximately 1 $cm^2$ and dehydrated at 55 – 60 $^oC$ for 7 – 8 h in a dehydrator (TSM Products, D10 – 32609, United States). The dehydrated pieces were ground in a tabletop laboratory grinder (Jaipan, India) and sieved through a laboratory sieve set (Ailmill, India) to make powder with particle size of 0.425 - 0.850 mm. Mango peel powder was packaged in metalized polyester bags and stored in air tight polypropylene containers at ambient conditions until further use.

All chemicals used in the study were analytical grade purchased from Merck Chemicals, India, VWR chemicals, USA and Sigma Aldrich, Germany. Ethanol (96%, v/v) was procured from Lanka Sugar Company (Pvt) Limited for extraction of pectin.

### Experimental design

A 3-factor-3-level Box-Behnken design of response surface methodology (RSM) was employed to design the experimental runs and investigate the effect of extraction conditions (pH, temperature and time) on yield and the DE of pectin. Fifteen experimental runs were carried out in triplicate represented by center points (Table 1). The conditions used were; pH of 1.3, 2.5 and 3.7, temperature of 60, 75 and 90 $^oC$ and time of 45, 90 and 135 min which were selected based on previous studies on various fruit peels (Kratchanova *et al.,* 2004; Kanmani *et al*., 2014; Müller-Maatsch *et al*., 2016). The experimental runs were conducted in a randomized order.

**Table 1. Box- Behnken Design of RSM employed for investigation on the effect of extraction conditions on yield and degree of esterification of pectin extracted from mango peel**

| Run No | Extraction conditions and levels | | |
|---|---|---|---|
| | pH | Temperature (°C) | Time (min) |
| 1 | 1.3 | 60 | 90 |
| 2 | 3.7 | 60 | 90 |
| 3 | 1.3 | 90 | 90 |
| 4 | 3.7 | 90 | 90 |
| 5 | 1.3 | 75 | 45 |
| 6 | 3.7 | 75 | 45 |
| 7 | 1.3 | 75 | 135 |
| 8 | 3.7 | 75 | 135 |
| 9 | 2.5 | 60 | 45 |
| 10 | 2.5 | 90 | 45 |
| 11 | 2.5 | 60 | 135 |
| 12 | 2.5 | 90 | 135 |
| 13 | 2.5 | 75 | 90 |
| 14 | 2.5 | 75 | 90 |
| 15 | 2.5 | 75 | 90 |

Run number was generated by Minitab 17 statistical software

**Extraction of pectin**

Pectin from mango peel powder was extracted according to the method described by Kratchanova *et al.* (2004). Approximately 10 g of mango peel powder was mixed with 300 ml of distilled water and pH was adjusted to required levels indicated in Table 1 using 1 M hydrochloric acid. The suspension was left for 20 - 30 min with occasional stirring for equilibrium. The pH was re-adjusted as required. Sample containers were partially covered with watch glasses and heated at predetermined temperatures for respective times (Table 1) in a water bath (Labtech, LWB-306DS). Subsequently, the hot suspension was filtered through a muslin cloth and cooled to 4 °C in an ice bath. An equal volume of 96% (v/v) ethanol was gradually added to the sample and slowly stirred for nearly 5 min. The solution was then allowed to rest for 1 h and the coagulated pectin was separated by filtering through a muslin cloth. Another equal volume of ethanol was added to the filtrate and coagulated pectin was separated as above. The isolated pectin was washed 5 times with 96% (v/v) ethanol, dried at 35 °C in an air convection incubator (Pol- Eko Aparatura – CLW 15, Poland) for overnight and weighed after cooled to ambient temperature. The dried sample was ground into fine powder and stored in airtight amber glass containers under ambient conditions until determination of the DE.

**Determination of yield**

The yield of pectin from mango peel was calculated by using the Equation 1.

$$\text{Yield (\%)} = \frac{W_o \, X \, 100}{W_1} \qquad \text{……..(1)}$$

where, $W_0$ is the weight of dried pectin before grinding and packaging (g) and $W_1$ is the weight of dried mango peel powder used for extraction (g).

**Determination of the DE**

According to Pasandide *et al.* (2015), the powdered sample (0.2 g) was added into a conical flask containing 20 ml of distilled water and 3 ml of 96% ethanol and thoroughly dissolved using a vortex mixer at 3000 rpm (Velp Scientifica – ZX3, USA). The solution containing few drops of phenolphthalein indicator was titrated against 0.1 M standardized sodium hydroxide ($V_1$). Then, 10 ml of standardized 0.1 M sodium hydroxide was added slowly to the solution while occasional swirling of the flask. After leaving the flask aside for 15 min, 10 ml of standardized 0.1 M hydrochloric acid was added and mixed well. The solution was titrated against 0.1 M standardized sodium hydroxide ($V_2$) using phenolphthalein as the indicator. The DE was calculated using the Equation 2.

$$\text{DE} = \frac{V_2 \times 100}{V_1 + V_2} \qquad \text{……..(2)}$$

**Statistical analysis of experimental data**

DE was estimated in duplicate and the average was used for modelling. Design of experiment and all statistical analysis were executed in Minitab 17 (Minitab Inc., State College, PA, USA). Multiple regression analysis was performed to develop an empirical quadratic second degree polynomial model. Experimental data were analyzed to fit the second degree polynomial equation (Equation 3) generated by Minitab 17.

$$Y = \beta_0+\beta_1X_1+ \beta_2X_2+ \beta_3X_3+\beta_{12}X_1X_2+ \beta_{13}X_1X_3+ \beta_{23}X_2X_3+ \beta_{11}X_1^2+ \beta_{22}X_2^2+ \beta_{33}X_3^2 \qquad \text{…(3)}$$

where,
Y - DE
$X_1$, $X_2$ and $X_3$ – pH, temperature and time
$\beta_0$ – Intercept coefficient
$\beta_1$, $\beta_2$, $\beta_3$ – Linear coefficient
$\beta_{12}$, $\beta_{13}$, $\beta_{23}$ – Quadratic coefficient
$\beta_{11}$, $\beta_{22}$, $\beta_{33}$ - Interaction coefficient

Analysis of variance (ANOVA) was used to determine the significance and accuracy of developed empirical quadratic second degree polynomial model and to analyze the effect of extraction conditions on DE of mango peel pectin.

## RESULTS AND DISCUSSION

### Yield of mango peel pectin

The conditions namely pH, temperature and time (combinations of 15 experimental runs) used for extracting pectin from mango peel affected the yield varied from 6.1 to 16.3% (dry weight basis).
Combination of pH 2.5, 90 ºC and 135 min resulted in the highest yield whereas pH 3.7, 60 ºC and 90 min resulted in the lowest yield (Table 2).

**Table 2. Yield of pectin extracted from mango peel under different combinations of extraction conditions using Box-Benkhen design**

| Run No | Extraction conditions | | | |
|---|---|---|---|---|
| | pH | Temperature (ºC) | Time (min) | Yield (%) |
| 1 | 1.3 | 60 | 90 | 11.5[a] |
| 2 | 3.7 | 60 | 90 | 6.1[b] |
| 3 | 1.3 | 90 | 90 | 11. 8[c] |
| 4 | 3.7 | 90 | 90 | 14.6[d] |
| 5 | 1.3 | 75 | 45 | 12.7[e] |
| 6 | 3.7 | 75 | 45 | 8.7[f] |
| 7 | 1.3 | 75 | 135 | 13.3[g] |
| 8 | 3.7 | 75 | 135 | 13.4[h] |
| 9 | 2.5 | 60 | 45 | 8.4[i] |
| 10 | 2.5 | 90 | 45 | 15.0[j] |
| 11 | 2.5 | 60 | 135 | 9.8[k] |
| 12 | 2.5 | 90 | 135 | 16.3[l] |
| 13 | 2.5 | 75 | 90 | 13.2[m] |
| 14 | 2.5 | 75 | 90 | 14.3[n] |
| 15 | 2.5 | 75 | 90 | 13.8[o] |

Means in the same column that do not share the same letter are significantly different

Multiple regression analysis of experimental data revealed that 17.1% of pectin could be extracted at pH 3.38, 90 ºC for 135 min. Pectin yield obtained in this study is in agreement with previous studies where pH 2.0, 82 ºC for 120 min resulted in 18.5% of pectin (Girma and Worku, 2016) and pH 1.5, 85 ºC for 60 min resulted in 12.0% pectin (Koubala *et al.,* 2008) from mango peel. Moreover, extraction at pH 2.0, 100 °C for 60 min resulted in 21.7% pectin from alcohol insoluble residue of mango peel (Patel, 2017). Yields of pectin extracted from mango peel are comparable with the same obtained from other commercially used plant materials such as citrus peel, apple pomace and sugar beet which yielded 18 - 30, 16.68 and 16.2%, respectively (Yapo *et al*., 2007; Liew *et al*., 2018; Wang *et al*., 2014). Thus, the present study revealed that fresh mango peel from processing industry could be successfully used for commercial scale pectin extraction in Sri Lanka.

### The DE of mango peel pectin

The presence of both HM pectin (DE > 50 %) and LM pectin (DE < 50 %) in the extracted mango peel pectin was evident (Table 3). Previous studies reported the presence of only HM pectin with DE ranging between 53 and 79% in mango peel pectin and the need for further de-esterification to obtain LM pectin (Berardini *et al*., 2005; Sirisakulwat *et al*., 2010; Geerkins *et al.,* 2015; Patel, 2017; Oleivera *et al.*, 2018). Such variations in the DE of pectin may probably be attributable to the differences in mango variety, ripening stage and extraction conditions. Nonetheless, the present study clearly showed that LM pectin can also be produced from mango peel by proper adjustment of extraction conditions (pH, temperature and time) without de-esterifying HM pectin, which is an addition in cost.

**Table 3. Degree of esterification of pectin extracted from mango peel under different extraction conditions using Box-Benkhen design**

| Run No | Extraction conditions | | | |
|---|---|---|---|---|
| | pH | Temperature (°C) | Time (min) | DE (%) |
| 1 | 1.3 | 60 | 90 | 45.5$^{o}$ |
| 2 | 3.7 | 60 | 90 | 87.5$^{a}$ |
| 3 | 1.3 | 90 | 90 | 45.7$^{n}$ |
| 4 | 3.7 | 90 | 90 | 75.0$^{j}$ |
| 5 | 1.3 | 75 | 45 | 54.4$^{l}$ |
| 6 | 3.7 | 75 | 45 | 87.1$^{b}$ |
| 7 | 1.3 | 75 | 135 | 51.9$^{m}$ |
| 8 | 3.7 | 75 | 135 | 85.4$^{c}$ |
| 9 | 2.5 | 60 | 45 | 83.3$^{e}$ |
| 10 | 2.5 | 90 | 45 | 83.9$^{d}$ |
| 11 | 2.5 | 60 | 135 | 81.8$^{f}$ |
| 12 | 2.5 | 90 | 135 | 71.4$^{k}$ |
| 13 | 2.5 | 75 | 90 | 76.9$^{i}$ |
| 14 | 2.5 | 75 | 90 | 77.8$^{h}$ |
| 15 | 2.5 | 75 | 90 | 78.8$^{g}$ |

Means in the same column that do not share the same letter are significantly different

### Regression model for DE of mango peel pectin

The empirical quadratic second degree polynomial model developed based on pH, temperature and time is as follows;

$$DE = -86.3 + 70.07\,pH + 1.977\,Temperature - 0.142\,Time - 8.665\,pH \times pH - 0.00912\,Temperature \times Temperature + 0.002140\,Time \times Time - 0.1708\,pH \times Temperature + 0.0039\,pH \times Time - 0.00405\,Temperature \times Time \quad \text{……..(4)}$$

F value (5.76) and P-value ($P$=0.000) of the model confirmed its significance and accuracy. Insignificant lack of fit ($P$=0.152) denoted that the model adequately fitted to experimental data. Coefficient of determination ($R^2$=99.48), adjusted $R^2$ (98.54), and predicted $R^2$ (92.41)

were close to 1, which further ensured the fitness of the model with experimental data (Table 4). The value of $R^2$ (99.48) indicated that 99.48 % total variation by pH, temperature and time can be explained by the model to predict DE with only 0.52% of total variation that could not be revealed. These findings confirmed that the model could be used to predict DE of mango peel pectin for given extraction condition.

**Table 4. Analysis of variance of regressed model for effect of extraction conditions on degree of esterification of pectin extracted from mango peel**

| Source | Contribution (%) | Adj SS | Adj MSS | *F*-Value | *P*-Value |
|---|---|---|---|---|---|
| Model | 99.48 | 3207.65 | 356.41 | 106.12 | **0.000** |
| Linear | 76.07 | 2452.81 | 817.60 | 243.43 | **0.000** |
| pH | 72.94 | 2351.97 | 2351.97 | 700.27 | 0.000 |
| Temperature | 1.83 | 59.14 | 59.14 | 17.61 | 0.009 |
| Time | 1.29 | 41.71 | 41.71 | 12.42 | 0.017 |
| Square | 21.31 | 687.02 | 229.01 | 68.18 | **0.000** |
| pH*pH | 18.50 | 574.86 | 574.86 | 171.16 | 0.000 |
| Temperature* Temperature | 21.12 | 15.53 | 15.53 | 4.63 | 0.084 |
| Time* Time | 2.15 | 69.36 | 69.36 | 20.65 | 0.006 |
| 2 –way interaction | 2.10 | 67.82 | 22.61 | 6.73 | **0.033** |
| pH* Temperature | 1.17 | 37.79 | 37.79 | 11.25 | 0.020 |
| pH*Time | 0.01 | 0.18 | 0.18 | 0.05 | 0.826 |
| Temperature* Time | 0.93 | 29.85 | 29.85 | 8.89 | 0.031 |
| Error | 0.52 | 16.79 | 3.36 | | |
| Lack-of-fit | 0.47 | 15.05 | 5.02 | **5.76** | **0.152** |
| Pure error | 0.05 | 1.74 | 0.87 | | |
| Total | 100.00 | | | | |
| $R^2$ | 99.48 | | | | |
| $R^2$ adjusted | 98.54 | | | | |
| $R^2$ predicted | 92.41 | | | | |

Adj SS - Adjusted sum square Adj MSS – Adjusted mean sum square

**The effect of pH, temperature and time on the DE**

pH (*P*=0.000) and time (*P*=0.030) exhibited significant main effects on the DE of mango peel pectin while temperature (*P*=0.080) did not. Interactive effects, which included linear effect of pH (*P*=0.000), temperature (*P*=0.009) and time (*P*=0.017), cubic effect of pH (*P*=0.000) and time (*P*=0.006) and quadratic effects of pH - temperature (*P*=0.020) and temperature - time (*P*=0.031) on DE were significant. However, cubic effect of temperature (*P*=0.084) and quadratic effect of pH - time (*P*=0.826) did not show significant effect (*P*=0.826) on DE of mango peel pectin. Similar findings were reported by other researchers for mango peel (Koubala *et al*., 2008; Faruque *et al*., 2016), durian peel (Wai *et al*., 2010) and banana peel (Emanga *et al*., 2008). The curvature in all 3D surface graphs (Figures 1, 2 and 3), demonstrated that the model carries quadratic effects of pH, temperature and time on DE that were statistically significant at P<0.050. Increase in DE was evident at higher level of pH and the highest was observed at upper left corner of the graph corresponds with the

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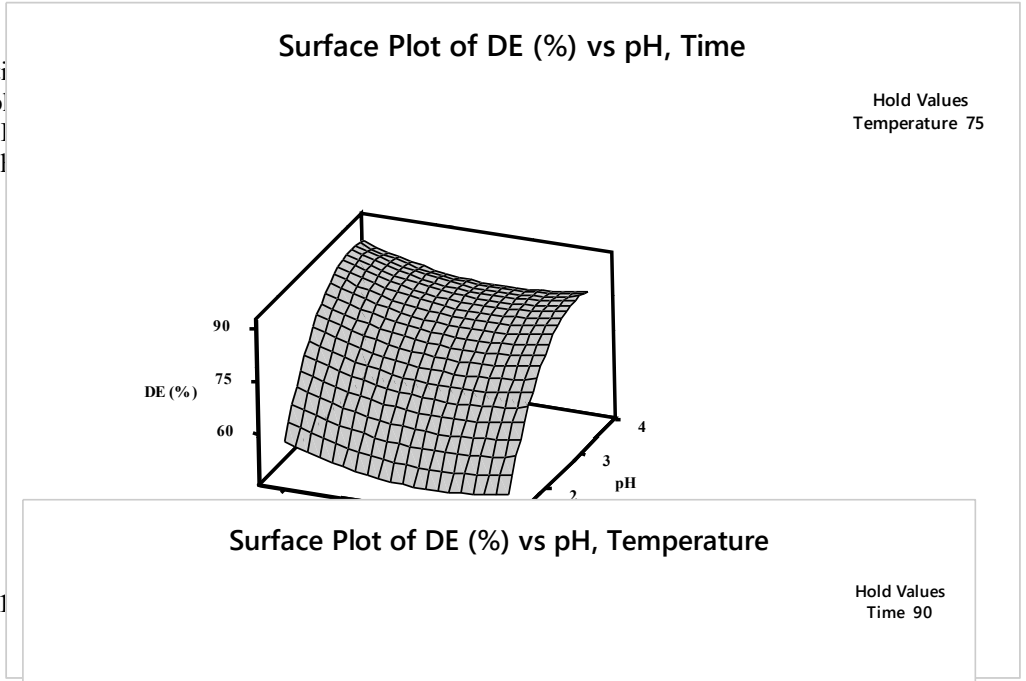


**Figure** 1

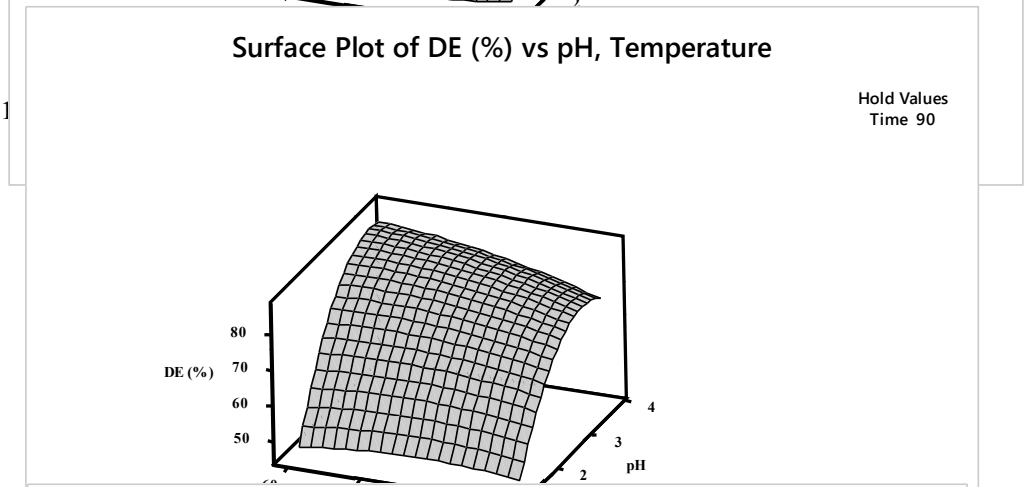


**Figure 2.**

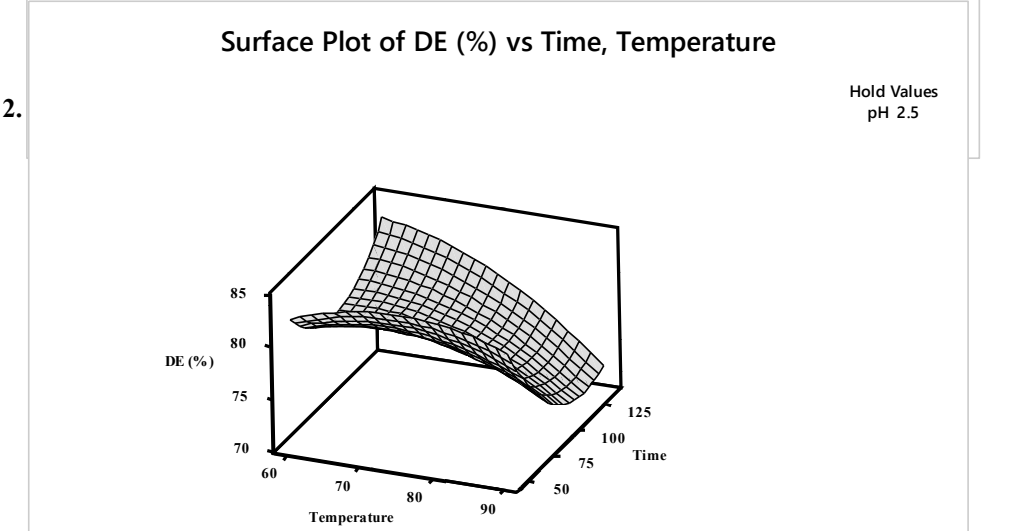


**Figure 3.**

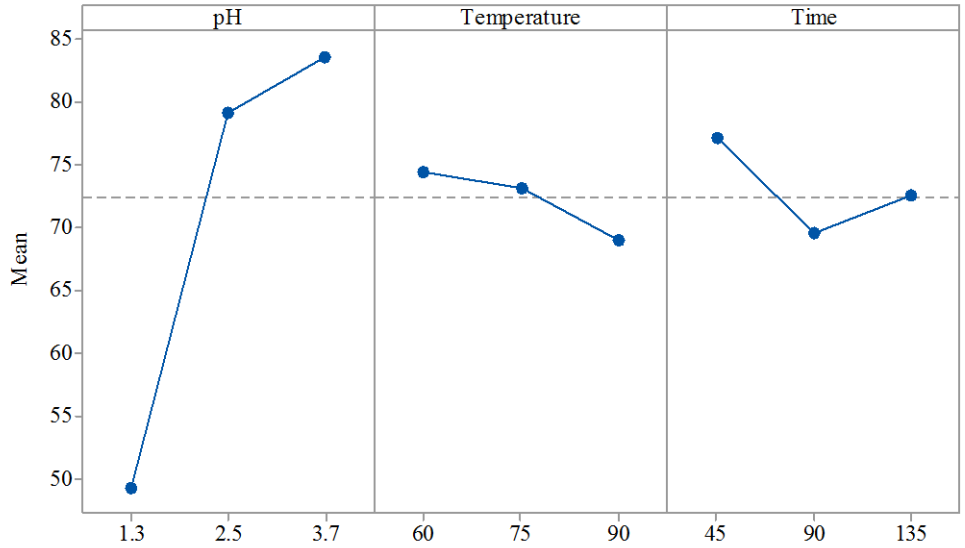


**Figure 4. Main effect of pH, temperature and time on degree of esterification of pectin from mango peel**

Main effects of pH, temperature and time on DE of mango peel pectin revealed direct impact of pH increments on DE (Figure 4). When the pH increases, inadequacy of $H^+$ ions to de-esterify pectin during extraction was indicated by steep ascent in DE up to pH 2.5 and moderate upsurge thereafter up to pH 3.7. With the rise in temperature, as more thermal energy is available to hydrolyze the ester bonds, further de-esterification of pectin during extraction was evident in this study. Similar results were reported in previous studies for peels of citrus (Kanmani *et al.*, 2014), mango (Sayah *et al.*, 2014) and banana (Emanga *et al.*, 2008). With increase in duration (time) of extraction, as the amount of thermal energy available to hydrolyze the ester bonds increases, de-esterification enhances, thereby reducing the DE of pectin (Adetunji *et al.*, 2017). However, reduction in the DE was evident during extraction up to 90 min and an increase thereafter up to 135 min. This may probably be due to the presence of other constituents such as carbohydrates, proteins and bioactive compounds in mango peels, which may possibly interfere with the extraction process.

## CONCLUSIONS

The main and interactive effects of pH, temperature and time significantly contribute to DE and yield of mango peel pectin. Mango peel could be a good commercial source for pectin production since it yielded 17.2% pectin that is comparable to other commercial sources such as apple pomace and sugar beet. The pH and time duration should be employed more carefully than temperature to obtain pectin with required DE since the effect of the former on DE is higher than that of the latter. The developed empirical quadratic second degree polynomial model can be used to predict the DE of mango peel pectin at commercial scale processing since the model was in good agreement with experimental data. Mango peel pectin with less than or more than 50% DE could be obtained when pH, temperature and time are manipulated according to the model. Hence, mango peel from fruit processing industries can be used as a single source to extract both HM and LM pectins, depending on their role in manufacturing of different foods whereas the commercial pectin resources used

at the present give only high methoxyl pectin and require an additional de-esterification process step to produce low methoxyl pectin.

## ACKNOWLEDGEMENT

The research grant of RG/2017/AG/2 by National Science Foundation, Sri Lanka is hereby gratefully acknowledged.

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