

***Chapter IV***  
***Results and Discussion***

## Chapter - IV

### RESULTS AND DISCUSSION

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This chapter includes the discussion of the observations and findings of the present study. The results as discussed in the order of the proposed objectives, beginning with the design of OH setup, heating behavior and energy performance of the OH process of different food materials. Further the mango puree is characterized on the base of acid and TSS content. Afterwards the optimization of OH process and enzyme and microbial inactivation kinetics is discussed. Finally the effects of optimized OH and conventional heating on various important parameters of mango puree are discussed followed by the storage study of treated mango puree at ambient conditions.

#### 4.1 Design and development of OH setup and its performance evaluation

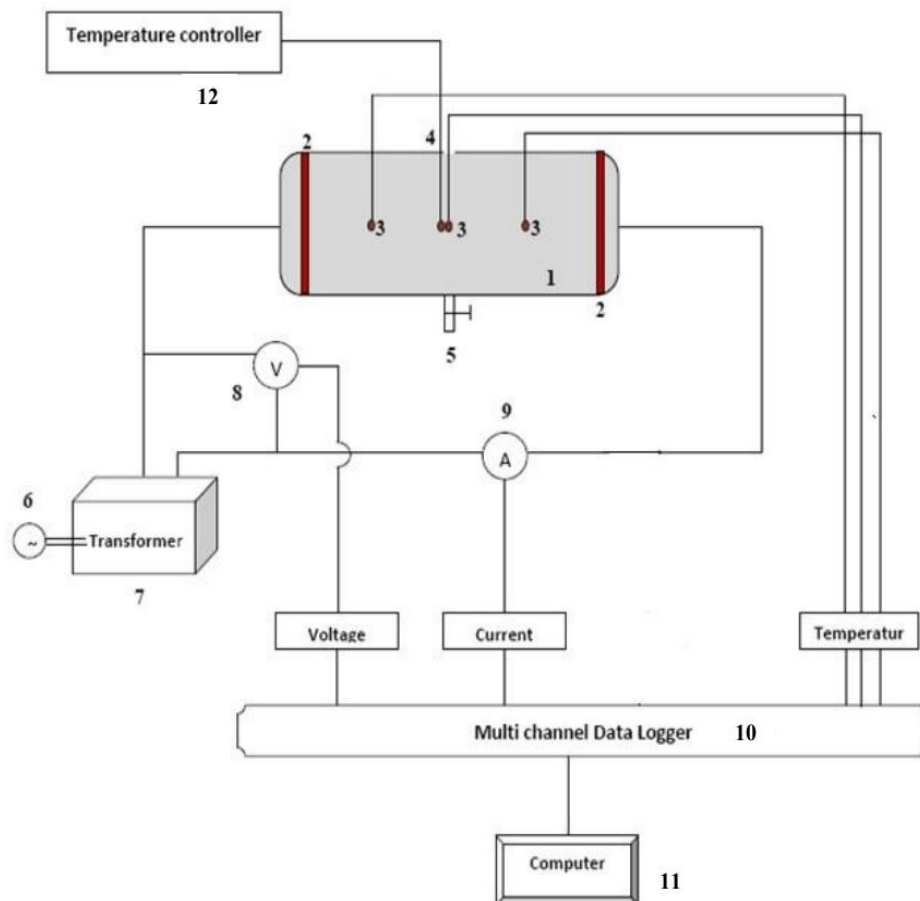
##### 4.1.1 Ohmic heating setup and its operation

The Ohmic heating setup as discussed in Chapter III at section 3.2.1 was consisted of a heating chamber, electrodes, variac transformer, domestic power supply and data acquisition assembly. The schematic diagram and the actual OH setup are shown in Fig. 4.1 and Fig. 4.2 respectively. The volumetric capacity of the heating chamber was  $50\pm 0.5$  mL, and the EFS range that could be adjusted was between 0-53 V/cm.

As per the description of the developed OH setup shown in Fig 4.1, the food material to be heated was filled through the opening (4). After filling the chamber, the temperature controller thermocouple (3) was placed at the geometrical centre of the heating chamber (1). The data logging assembly was connected to the electric circuit to collect the data of voltage (8), electric current (9) and to control the temperature the temperature controller (12) was also connected with the data logger. As the setup was ready, the power supply of the desired EFS was supplied to food material through electrodes. The temperature was raised as per the electrical conductivity (EC) of the food material and the EFS. The raise in temperature had a positive relationship with the EC and EFS. During the OH experiments as the target temperature was achieved it was maintained ( $\pm 1^\circ\text{C}$ ) by switching off the power supply controlled by a relay-based controller.

The performance of the OH process was evaluated with a motive to study the heating behavior of the fruits with different characteristics such as acid content, TSS, pH

and consistency. Thus, five different products viz mango puree, tomato juice, watermelon juice, pineapple juice and litchi juice were chosen for the evaluation of performance of the OH process. Conventionally thermal processing is the most common preservation method for the listed food materials. Therefore, the present objective will be discussing the OH behavior and energy efficiency of OH process at different EFS.



**Fig. 4.1** The schematic diagram of ohmic heating assembly which include (1) Heating chamber (2) Electrode (3) Thermocouple (4) Feed inlet (5) Product outlet (6) Power supply (7) Variac transformer (8) Voltmeter (9) Ammeter (10) Multi channel data logger (11) Computer (12) Temperature controller

#### 4.1.2 Properties of fresh fruit juice/puree

Some properties of fresh juice/ puree of selected fruits was measured and presented in Table 4.1. The acidity of the mango puree and litchi juice was found to be similar to each other and maximum among all the materials as mentioned in Table 4.1. The titratable acidity (TA) of watermelon juice was found to be minimum (0.21 %). The maximum pH (4.8) and minimum pH (3.9) of selected fruits was for mango puree and tomato juice respectively. Refractometric measurements revealed that tomato juice

contain minimum soluble solids of 4.3 °Brix, however the maximum 20 °Brix TSS was observed to be in mango puree. The density of pine apple juice was found close to that of water i.e. 0.988 kg/m<sup>-3</sup> and minimum among the samples.

**Table 4.1 Properties of the food samples studied in the present study**

| Raw Material     | Titratable Acidity<br>(% Citric acid) | Moisture<br>Content (%) | pH        | TSS<br>(° Brix) | Density<br>(kg/m <sup>3</sup> ) |
|------------------|---------------------------------------|-------------------------|-----------|-----------------|---------------------------------|
| Watermelon juice | 0.21±0.04                             | 88.60±0.14              | 5.40±0.18 | 9.50±0.30       | 1030±5.0                        |
| Tomato juice     | 0.43±0.02                             | 91.20±1.60              | 3.90±0.09 | 4.30±0.10       | 1090±7.0                        |
| Pineapple juice  | 0.48±0.03                             | 87.90±0.43              | 4.30±0.13 | 8.00±0.20       | 990±2.0                         |
| Litchi juice     | 0.49±0.07                             | 83.30±0.21              | 4.16±0.12 | 14.60±0.10      | 1050±3.0                        |
| Mango puree      | 0.50±0.06                             | 79.20±0.30              | 4.80±0.17 | 16.50±0.00      | 1080±9.0                        |



(a)



(b)

**Fig. 4.2 The actual lab scale ohmic heating setup (a) at Tezpur University (home institute) (b) University of Surrey (host institute)**

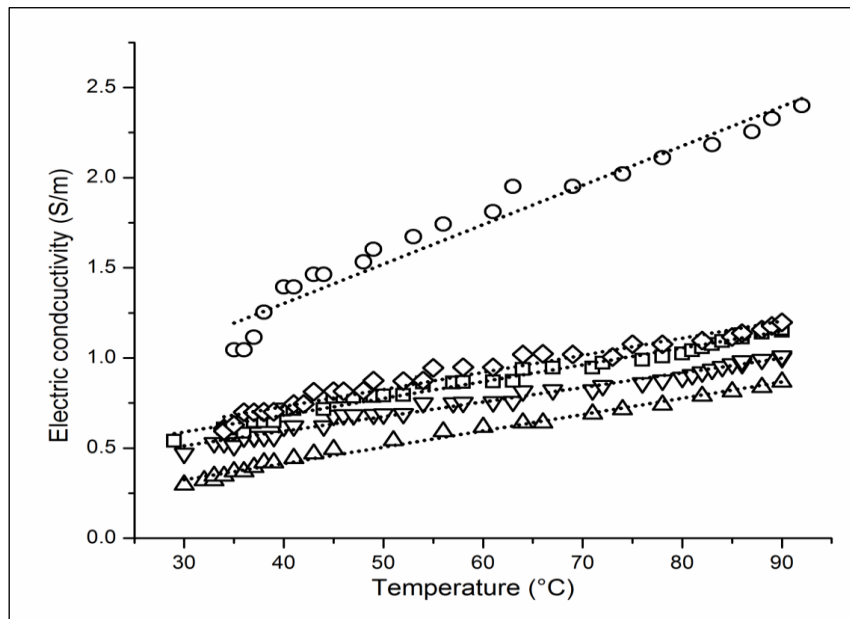
### 4.1.3 Ohmic heating behavior of fresh juice and puree

Ohmic heating uses an electric current passed through a sample, and due to the resistance of the sample, heat is produced increasing its temperature, when an electric current is applied to a food material by electrolytic conduction, collisions of mobile ions with other molecules transfers momentum to these molecules, thereby increasing their kinetic energy and heating the product [61]. The OH behavior is determined by many factors such as the electrical conductivity (EC) electric field strength (EFS) and some other intrinsic properties (e.g. acid content, consistency, sugar content) of the material being heated. Electrical conductivity actually helps the current to flow and electrical resistance to these current results in temperature rise. The EFS determines the amount of induced current hence the rate of heating of the material.

#### 4.1.3.1 Electric conductivity (EC) and OH behavior

Electric conductivity (EC) of a specific material depends upon the amount of ionic concentration of the food material, therefore, during OH all the samples heated according to their EC, the sample with lower EC heated slowly compared to that of having higher EC. Thereof, the heating curve of specific food material followed a trend directly related with the EC values of respective material (Fig. 4.3). The EC at room temperature ( $25 \pm 2$  °C) varied among the selected fruits, watermelon juice was found to have maximum EC among the selected fruits evaluated in present study, followed by pineapple juice, tomato juice, mango puree and litchi juice (Table 4.2) which infers that watermelon juice contained the highest and litchi juice lowest amount of charged ionic content relatively. Sarang et al. [91] reported slightly lower range of EC in fruit cuts (Apple, Strawberry, pear, pineapple), the possible reason for variation could be because of the state of material, they have measured the EC in solid cut fruit whereas values reported in present study are for the liquid (juice or puree). Electric conductivity increased linearly with the temperature in all the samples during OH as depicted in Fig. 4.3. The regression constant (m) and  $R^2$  value are showed in Table 4.2. Linear change in EC with increasing temperature during OH have been previously reported in many food products such as in apple and sour cherry juice [41], carrot and starch mixtures [113], cream having different percentage of fat content [53] and orange juice [43]. As reported by Icier and Ilicali [43] no specific trend was observed in EC change with the electric field strength (EFS). Additionally, in present study no major difference in EC was witnessed at different EFS; therefore, the results are reported as the average value of EC at 10-40 V/cm EFS. The

regression analysis showed that the inclination of the change of EC with temperature in litchi juice was observed relatively very high ( $m=0.218$ ) whereas in rest of the samples it was found relatively lower and also very much close to each other ( $m=0.0081-0.0095$ ). These results could be hypothesized that the excitement of ions was relatively higher in litchi juice with increasing the temperature, and further research at ionic structure level is required to validate the hypothesis.



**Fig. 4.3 Electric conductivity and temperature relationship of mango puree ( $\nabla$ ), tomato ( $\square$ ), pineapple ( $\diamond$ ), watermelon ( $\circ$ ) and litchi ( $\triangle$ ) juice during OH**

**Table 4.2 Electrical conductivity (EC) at room temperature and linear regression factors of change in EC with temperature**

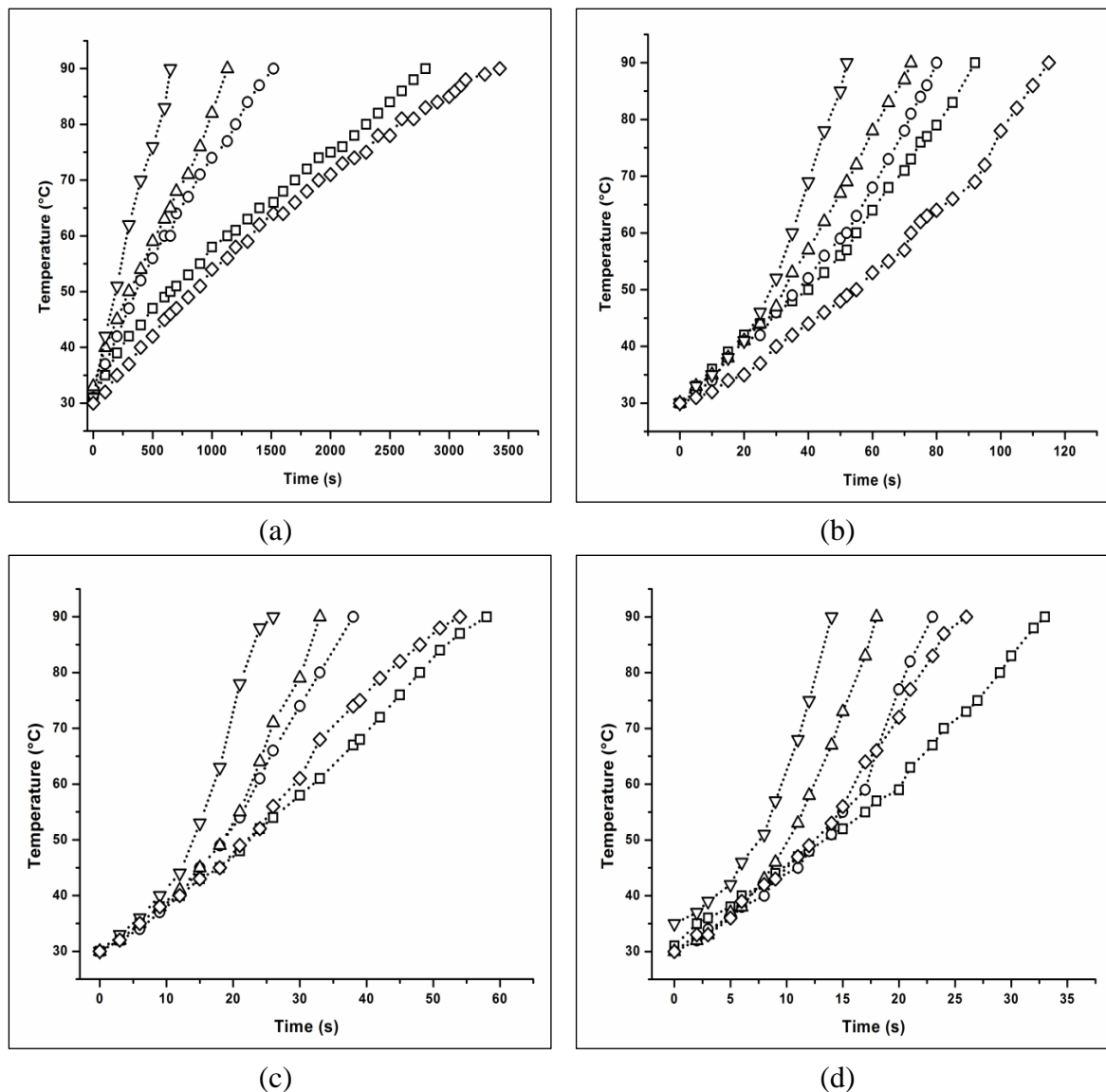
| Sample           | EC (S/m) at $25\pm 2$ °C | Coefficient ( $m \times 10^{-3}$ ) | $R^2$ |
|------------------|--------------------------|------------------------------------|-------|
| Mango puree      | 0.475                    | 8.1                                | 0.981 |
| Tomato juice     | 0.544                    | 9.3                                | 0.980 |
| Pineapple juice  | 0.589                    | 9.5                                | 0.957 |
| Watermelon juice | 0.974                    | 21.8                               | 0.952 |
| Litchi juice     | 0.278                    | 9.0                                | 0.989 |

#### 4.1.3.2 Electric Field Strength (EFS) and Ohmic Heating (OH) behavior

The heating rate of food was also observed to depend on EFS. It is evident for the data that at 10 V/cm, the water melon juice took 10 min to reach 90 °C and the litchi juice took 57 min. The heating rate increased drastically when the EFS was increased up to 20

V/cm or further. At 40 V/cm the heating was extremely rapid and it took only 0.23 and 0.55 min to reach 90 °C in watermelon juice and mango puree respectively, Icier and Ilicali [43] have also reported similar changes in OH behavior of peach and apricot puree while heating by applying 20-70 V/cm. Hence the heating history and temperature of OH system could be controlled by the EFS of power supply.

Litchi juice showed minimum EC and heated with slower rate as compared to mango puree which showed slightly higher EC (Table 4.2 and Fig. 4.3) at 10-20 V/cm as shown in Fig. 4.4 (a) and (b). However, the heating curve showed some interesting results, as the EFS was increased to 30 and 40 V/cm, the rate of heating in mango puree gets slower than that of litchi juice, which can be seen in Fig. 4.4 (c) and (d).



**Fig. 4.4** Ohmic heating behavior of mango puree (□), tomato juice (○), pineapple juice (△), watermelon juice (▽) and litchi juice (◇) at (a) 10, (b) 20, (c) 30 and (d) 40 V/cm EFS

These results are in accord with Icier and Ilicali [41], they reported that the rate of OH slows down as the solid concentration increased in apple and sour cherry juice concentrate. Figure 4.5 indicates that the rate of heating switched in between Mango puree and litchi juice samples which confirm that the viscosity or consistency of the material also has an important role in addition to the electrical conductivity of the food samples at higher EFS (30-40 V/cm in present study). Fryer et al. [30] has reported that the viscosity of the material has a role in temperature differences during OH, insignificant temperature differences can be observed in the food model having lower viscosity ( $1 \times 10^{-3}$  Pa.s) whereas at higher viscosity ( $800 \times 10^{-3}$  Pa.s), significant variation in temperature was observed.

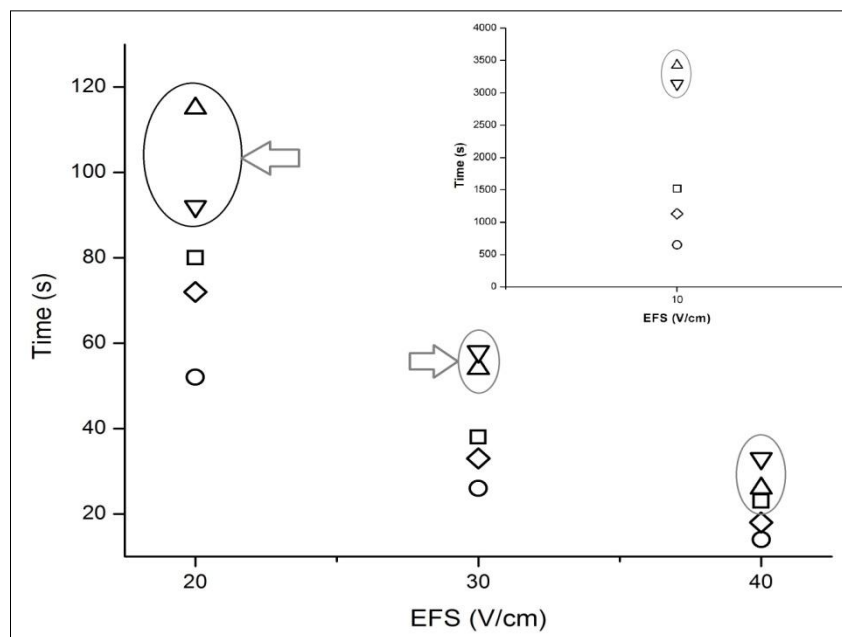
The rate of change of temperature (heating rate) was observed to follow the first order Eq. (4.1) increase with the EFS during OH in all the selected fruit materials. The results are depicted in Fig. 4.6 where as the model parameters are mentioned in the Table 4.3. The value of determination coefficient ( $R^2$ ) of 0.923-0.976 among the samples indicates the goodness of the fit.

$$\text{Heating rate} = HR_0 \exp(k \cdot v) \tag{Eq. (4.1)}$$

Where

$HR_0$  is the initial heating rate ( $^{\circ}\text{C}/\text{min}$ )

'k' is a constant and 'v' the electric field strength (V/cm)

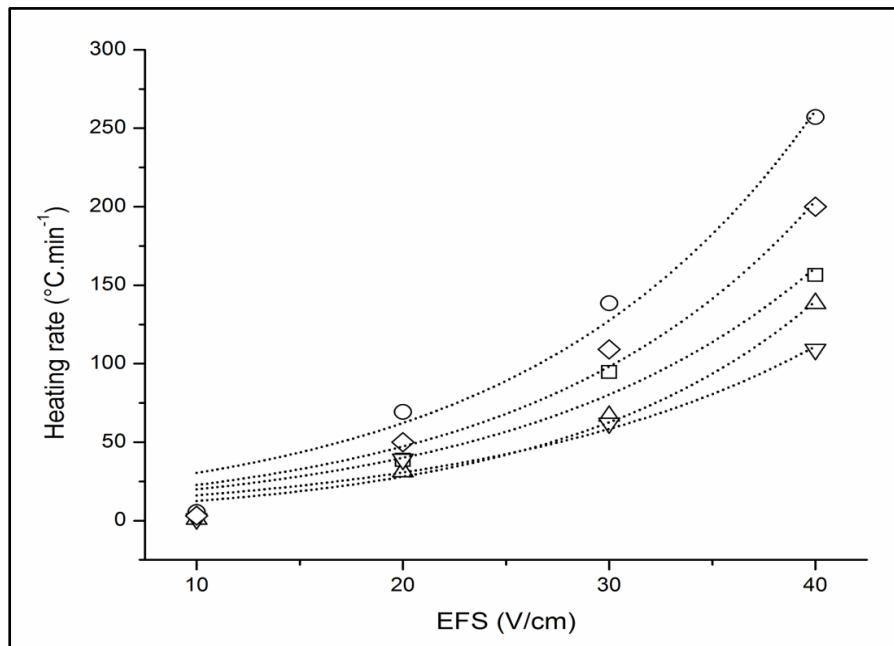


**Fig. 4.5** The time required to achieve the desired temperature by mango puree (▽), tomato (□), pineapple (◇), watermelon (○) and litchi (△) juice at different EFS of



**Table 4.3 Exponential model parameters of increase in heating rate with EFS**

| Fruit sample      | HR <sub>0</sub> | k     | R <sup>2</sup> |
|-------------------|-----------------|-------|----------------|
| Mango puree       | 8.499           | 0.064 | 0.923          |
| Tomato juice      | 9.939           | 0.069 | 0.940          |
| Pineapple juice   | 10.959          | 0.073 | 0.963          |
| Water melon juice | 14.830          | 0.071 | 0.965          |
| Litchi juice      | 5.627           | 0.080 | 0.976          |

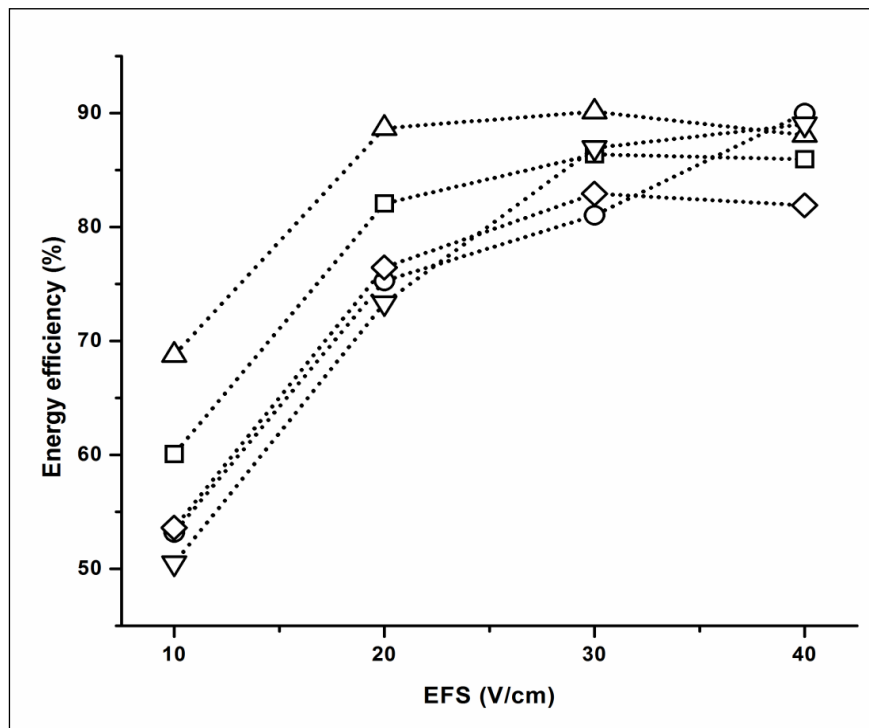


**Fig. 4.6 Exponential model (.....) and heating rate at different EFS in mango puree (▽), tomato (□), pineapple (◇), watermelon (○) and litchi (△) juice during OH**

#### 4.1.3.3 Performance of ohmic heating

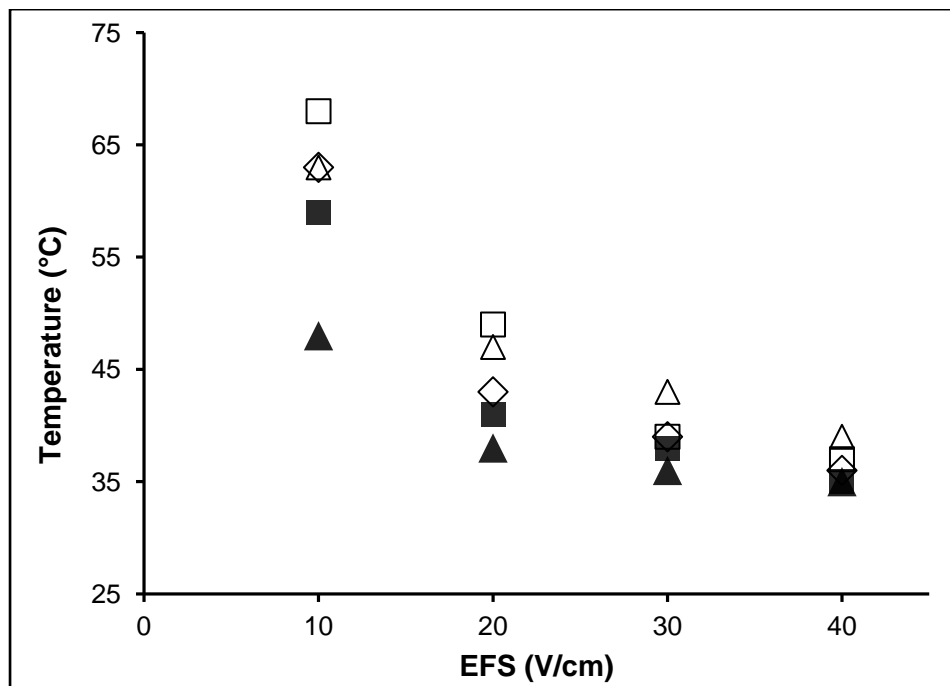
The actual or ideal amount of the energy required to heat 50 mL of every sample was calculated from the Eq. 3.7. The energy required to heat the samples ranged from 11.86 to 13.36 kJ/50 mL. The maximum energy requirement was calculated for tomato juice followed by watermelon, litchi, mango puree and pineapple juice, the results are illustrated in Table 4.4. The energy efficiency was calculated considering the heat capacity at initial and final temperature and also with an assumption that 99 % of electrical power gets utilized for heat generation; therefore, the energy efficiency of the OH process of the different samples was calculated by Eq. 3.10. The thermal or heating process cannot be perfectly efficient due to the heat loss through the heating surface to the environment. In present study the heat loss occurred through the surface of the heating chamber. However during OH the performance of different food materials varied with EFS and among the samples as well. The power (electrical energy) consumed at 10 V/cm

was extremely high as reported in Table 4.4, because the time required to achieve the target temperature of 90 °C was too long (Fig. 4.5), the slow heating results more amount of heat loss to the atmosphere by conduction through the heating chamber body. The efficiency of OH process at various EFS is shown in Fig. 4.7, from the figure it is evident that at 10 V/cm, more than double of theoretical energy (Eq. 3.7) was consumed for heating samples, thus making the heating process low energy efficient (<50 %). Cokgezme et al. [22] and Bozkurt and Icier [15] also reported lower energy efficiency of OH process at lower EFS for evaporation process of pomegranate juice and for cooking of beef respectively. As the EFS was increased above the 10 V/cm the power required for heating all the food samples get reduced sharply, due to rapid heating. Darvishi et al. [25] also reported the energy efficiency in the range of 67 and 87 % while applying 6 to 16 V/cm EFS during evaporation of tomato juice by OH. The temperature at the outer surface of the heating cell corresponding to the product temperature at 90 °C is shown in Fig. 4.8, the maximum outer surface temperature was achieved at 10 V/cm due to lower heating rate, and as the EFS was increased the heating rate also increased hence, resulted in lower outer surface temperature. This indicates that at lower EFS the heat loss was more thus, resulted in the poor efficiency of the OH process at lower EFS.



**Fig. 4.7** Energy efficiency (%) of the OH process of mango puree (◇), tomato juice (□), pineapple juice (○), watermelon juice and (△) litchi juice (▽)

The amount of heat energy consumed by heating cell to raise its temperature is reported in Table 4.4. The energy efficiency increased to 73.32 % in litchi juice and to 90.11 % in watermelon juice while increasing EFS from 20 to 30 V/cm. However, on further increase of EFS to 40 V/cm the energy efficiency remained stagnant in tomato juice and mango puree, whereas decreased slightly in watermelon juice. The energy efficiency of OH process in litchi juice and pineapple juice showed further increase as the EFS was increased from 30 to 40 V/cm. Icier and Ilicali [43] suggested that a portion of electrical energy may get utilized in physical, chemical and electro-chemical changes of product during OH, therefore similar changes might have caused reduction in energy efficiency in OH of litchi and watermelon juice as the EFS was increased from 30 to 40 V/cm.



**Fig. 4.8 Average temperature ( $T_f$ ) of heating chamber at the time of final temperature of mango puree ( $\Delta$ ), tomato juice ( $\diamond$ ) pineapple juice ( $\blacksquare$ ) watermelon juice ( $\blacktriangle$ ) and litchi juice ( $\square$ ) at different EFS**

The above results indicated that OH process has a great potential to replace the conventional thermal processing. As the efficiency of more than 90 % is achievable, the loss of energy can be minimized thus, can be the processing cost. From the above results it was observed that the physico-chemical properties such as acidity and consistency of the food material define its OH behavior. And the prime objective of this study was to verify the possibility of OH as an alternative thermal processing method of mango puree. Therefore, before optimizing the OH parameters for mango puree, it was important to

standardize or characterize the mango puree on the base of two important parameters i.e. acid content and TSS. Both the two parameters are very important while making any fruit product such as juice, puree etc. The characterization of mango puree is discussed in the next section.

**Table 4.4 Energy consumption and losses during OH at different EFS**

| Sample           | mC <sub>p</sub> dT<br>(kJ) | Electrical energy supplied<br>(kJ) |            |            |            | Amount of heat loss (kJ)<br>through chamber |            |            |            |
|------------------|----------------------------|------------------------------------|------------|------------|------------|---|------------|------------|------------|
|                  |                            | 10<br>V/cm                         | 20<br>V/cm | 30<br>V/cm | 40<br>V/cm | 10<br>V/cm                                  | 20<br>V/cm | 30<br>V/cm | 40<br>V/cm |
| Tomato juice     | 13.36                      | 26.75                              | 17.94      | 16.74      | 16.85      | 9.85  | 3.88       | 2.69       | 1.79       |
| Pineapple juice  | 11.86                      | 27.01                              | 17.52      | 15.89      | 13.78      | 8.66  | 3.28       | 2.39       | 1.49       |
| Watermelon juice | 12.47                      | 21.59                              | 15.42      | 15.08      | 15.57      | 5.37  | 2.39       | 1.79       | 1.49       |
| Litchi juice     | 12.29                      | 31.01                              | 19.56      | 15.59      | 15.09      | 11.34                                       | 5.67       | 2.69       | 2.09       |
| Mango puree      | 12.19                      | 28.22                              | 18.10      | 16.25      | 16.52      | 9.85  | 5.08       | 3.88       | 2.69       |

#### 4.2 Characteristics of fresh mango puree at different levels of Acid and TSS content

The puree obtained from fresh and fully ripe mango fruit was analyzed for different physico-chemical parameters listed in Table 4.5. The titratable acidity and pH was found to be 0.49% and 3.89 respectively. The fresh puree was found to contain TSS of 15.5 °B, Kaushik et al. [50] also reported similar observations. The water activity of the mango puree was found to be slightly lower than pure water whereas the density was observed to be slightly higher than pure water respectively. The color parameters ‘a\*’ (green-red) and ‘b\*’ (blue-yellow) suggested the rich reddish-yellow color of the mango puree.

**Table 4.5 Characteristics of fresh mango puree**

| Parameter                          | Value      |
|------------------------------------|------------|
| Titratable acidity (% citric acid) | 0.49±0.00  |
| TSS (° Brix)                       | 15.5±0.50  |
| pH                                 | 4.1±0.20   |
| Color parameters                   |            |
| L*                                 | 36.12±0.30 |
| a*                                 | 41.80±0.60 |
| b*                                 | 11.21±0.30 |
| Density (kg.m <sup>-3</sup> )      | 1062±12    |
| Water activity                     | 0.99±0.01  |

The effect of three levels of acid and TSS content on various parameters of the mango puree was studied as discussed below.

#### 4.2.1 pH and water activity

The water activity and pH are two important parameters for microbial and enzymatic action, thus shelf life of the fruit products. The changes in water activity and pH of mango puree due to increasing acid (0.50-0.62 g/100g) and sugar (20-24 °Brix) are reported in Table 4.6. The addition of acid caused a significant change in the pH, however, pH showed no change while increasing the TSS from 20 to 24 °B. Reduction of the pH turns out as a hurdle for the microbial growth in mango puree; Guerrero-Beltrán et al. [34] observed that addition of acid enhanced the microbial reduction. The water activity of mango puree increased slightly with increase in acid content and decreased with the TSS. Gracia-Martinez et al. [31] and Guerrero and Alzamora [33] also have observed the reduction in water activity by increasing the TSS of the orange and kiwi fruit products. These observations signify that the added sugar binds the free water present in food material hence, makes it unavailable or reduces the water activity. However addition of acid released some water present in the matrix of the fruit material, thus, accounts for increasing the water activity. The fruit purees act as pectin, water and sugar gels, where water and pectin play an important role in the networking for the stability of the gel [90]. The gel networks are generally made by the ordered conformation and associations between the pectin-sugar, thus causing the water to get trapped in between, and this networking is highly sensitive to the pH change caused by the addition of acid [23].

**Table 4.6 Effect of Acid content and TSS on pH and water activity of mango puree<sup>x</sup>**

| TSS (°Brix) | Acid content (%) | pH                     | Water activity         |
|-------------|------------------|------------------------|------------------------|
| 20          | 0.50             | 5.07±0.07 <sup>a</sup> | 0.98±0.00 <sup>a</sup> |
| 20          | 0.56             | 4.75±0.03 <sup>b</sup> | 0.99±0.00              |
| 20          | 0.62             | 4.53±0.01 <sup>c</sup> | 0.99±0.00 <sup>b</sup> |
| 20          | 0.50             | 5.07±0.07 <sup>a</sup> | 0.99±0.00 <sup>b</sup> |
| 22          | 0.50             | 5.09±0.04 <sup>a</sup> | 0.98±0.00 <sup>a</sup> |
| 24          | 0.50             | 5.13±0.01 <sup>a</sup> | 0.98±0.00 <sup>a</sup> |

<sup>x</sup> Values reported as mean±SD, values in the same column with different alphabet were significantly different at (p<0.05)

The instability of the gel at higher acid content might have resulted in the release of some water from the pectin-water-sugar network thus, caused the water activity to

increase. Further the addition of sugar and acid might have brought the variations in the solubility and interaction with other major components therefore resulted in the changes in water activity.

#### 4.2.2 Back extrusion Parameters

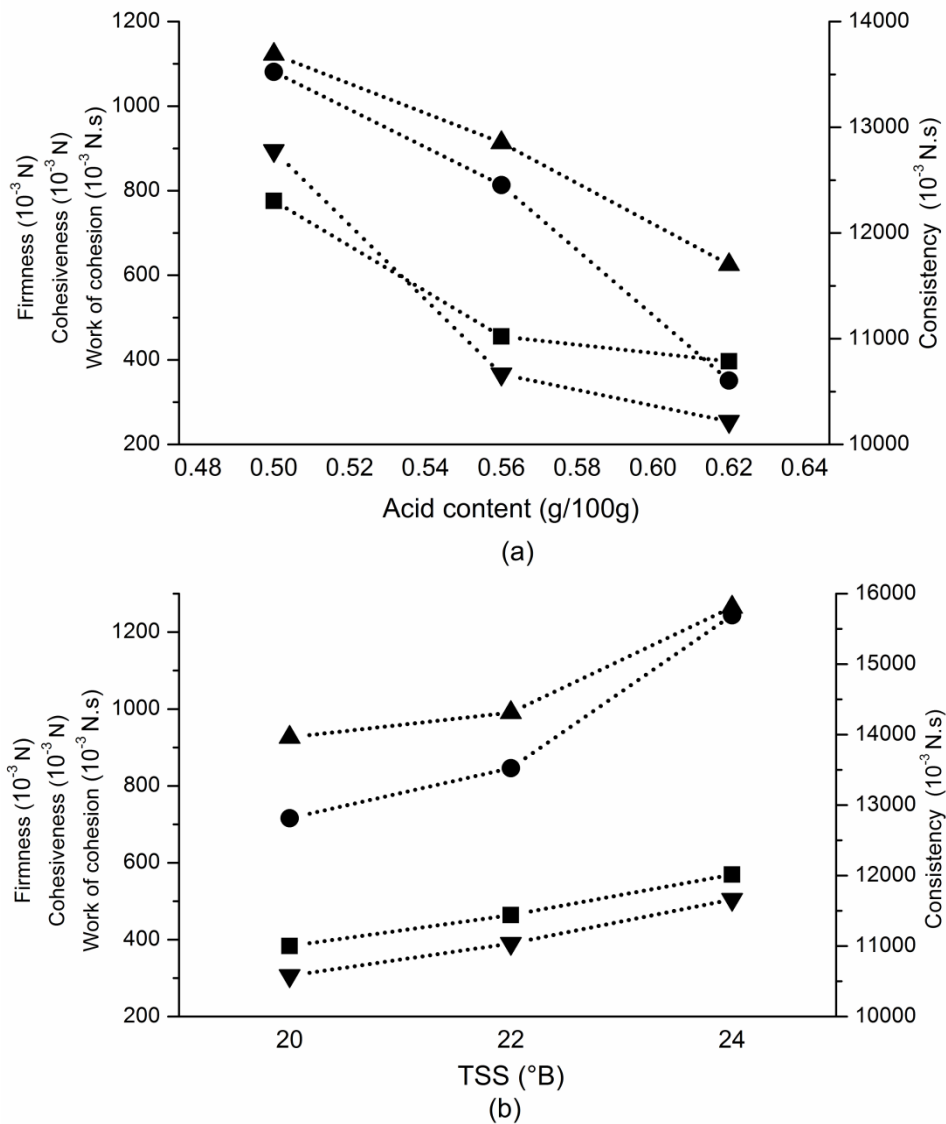
The most of the fruits puree and pulp have complex textural behaviour due to the interaction in between soluble solids, pectic substances and suspended solids [91]. The change in acid content and TSS of the mango puree has shown some interesting results when analyzed for the back-extrusion (textural) parameters viz work of cohesion ( $W_C$ ), firmness, consistency, and cohesiveness. The back extrusion parameters showed a positive relationship with increase in TSS, while as negative with that of acid content. Sanchez et al. [90] reported that complex interactions among soluble sugars, pectic substances and suspended solids result in the change of the rheological properties of the food material. The  $W_C$  is the work required to produce droplet from a liquid sample during spraying, therefore higher value of work of cohesion means it is difficult to spread the material. It was observed to decrease with increasing the acid content; hence the spreadability of the puree can be considered to increase with the acid. However, the  $W_C$  was found to increase with the addition of the sugar. The change in pH due to acid addition would have destabilized the sugar-water-pectin network, therefore resulted in the reduction of WOC (Fig. 4.9a), whereas sugar addition increased the cohesion and water binding, therefore resulted  $W_C$  increase (Fig. 4.9b).

The  $W_C$  was increased from 0.92 to 1.26 N.s when the TSS was increased from 20 to 24 °B. The firmness of mango puree was found to be reduced from 0.75 to 0.40 N when the acid content was increased from 0.50 to 0.62 g/100g, respectively, whereas the increase in TSS resulted in its increase from 0.39 to 0.57 N. A similar trend was also observed in the consistency and cohesiveness of the mango puree by changing its acid content and TSS as shown in Fig. 4.9. The change in consistency involves the interaction between pectic substances of the materials with the added sugar and acids. Guerrero and Alzamora [33]; Singh and Eipeson [103] have also reported that the addition of sugar increases the consistency of mango puree and concentrated mango juice respectively.

#### 4.2.3 Rheological characteristics of mango puree

Rheological behaviour of fruit purees are of substantial interest for the development fruit products for technological and marketing reasons. It is a reliable tool for understanding

molecular structure changes during the preparation and processing of the product. Thus it is important for the process optimization and equipment design as well as the final product [12, 33]. The rheological parameters of the mango puree are presented in Table 4.7, the values of storage modulus ( $G'$ ) were higher than that of loss modulus ( $G''$ ) as shown in Fig 4.10, it signifies the elastic components dominated the viscous components which another way indicates the more gel-like structure than viscous behavior, Gundurao et al., [35] also reported the same observations while analyzing mango puree. The phase angle values recommend that the mango puree exhibits visco-elastic nature. The complex viscosity was found to reduce exponentially with the frequency in all the samples at both temperatures 30 and 90 °C (Fig. 4.11).



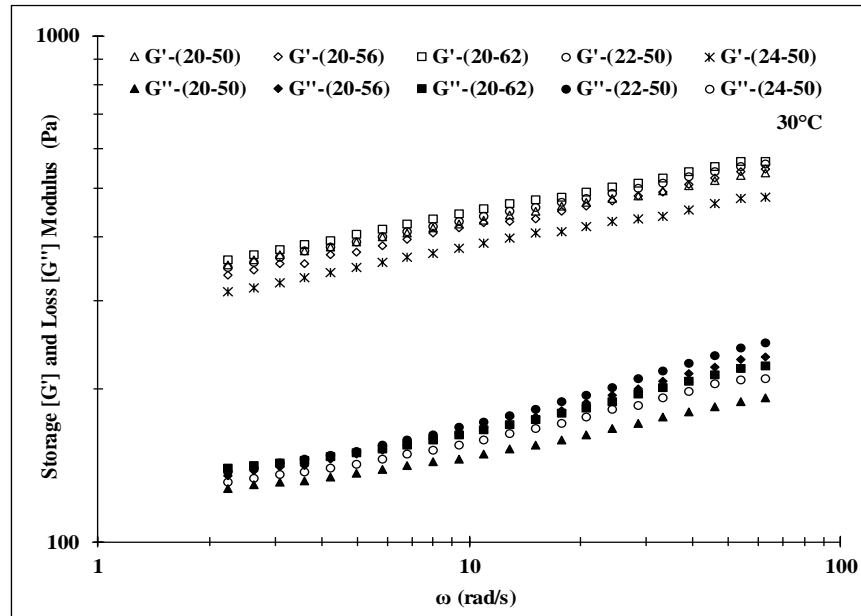
**Fig. 4.9 Back extrusion parameters, Firmness (■), Consistency (●), Cohesiveness (▼) and work of cohesion (▲) of the sample with different (a) acid and (b) TSS level**

**Table 4.7 Rheological characteristics of the samples at 30 and 90 °C temperature (at 1-Hz)**

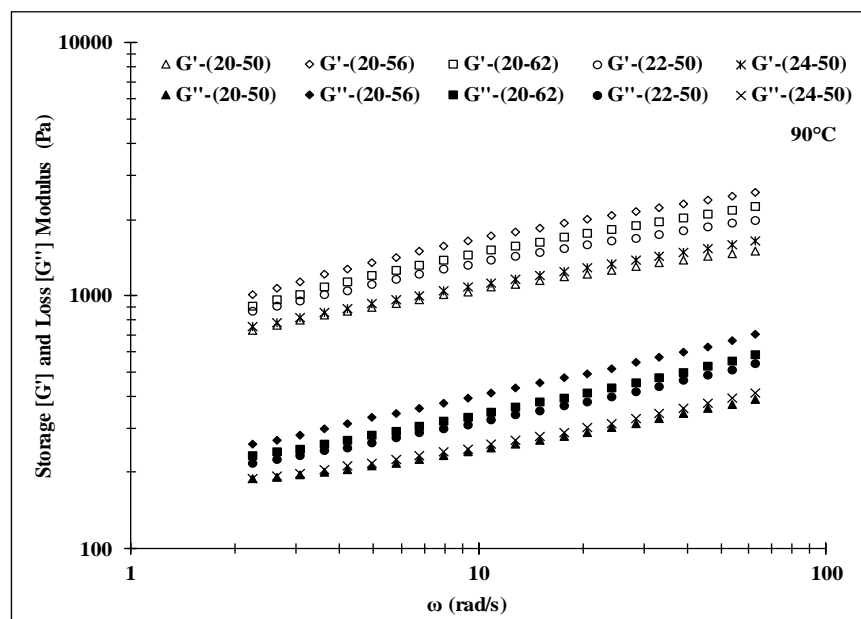
| TSS          | Acid content | Storage Modulus<br>G' (Pa) | Loss modulus<br>G'' (Pa) | Complex viscosity<br>$\eta^*$ (Pa.s) | Phase angle<br>$\delta$ |
|--------------|--------------|----------------------------|--------------------------|--------------------------------------|-------------------------|
| <b>30 °C</b> |              |                            |                          |                                      |                         |
| 20           | 0.50         | 408.4±01.8                 | 145.2±4.9                | 63.5±0.5                             | 19.6±0.5                |
| 20           | 0.56         | 396.0±08.5                 | 155.3±7.5                | 62.6±1.5                             | 20.1±0.6                |
| 20           | 0.62         | 425.0±12.7                 | 155.7±9.3                | 66.6±2.2                             | 21.4±0.5                |
| 20           | 0.50         | 408.4±01.8                 | 145.2±4.9                | 63.5±0.5                             | 19.6±0.5                |
| 22           | 0.50         | 410.0±26.9                 | 151.8±8.2                | 64.4±4.1                             | 20.3±0.6                |
| 24           | 0.50         | 362.7±12.6                 | 156.0±18.4               | 58.0±1.0                             | 23.3±1.0                |
| <b>90 °C</b> |              |                            |                          |                                      |                         |
| 20           | 0.50         | 1074.6±18.5                | 239.3±7.0                | 161.6±3.2                            | 12.6±0.1                |
| 20           | 0.56         | 1465.2±54.4                | 328.7±16.0               | 220.7±8.3                            | 12.6±0.1                |
| 20           | 0.62         | 1856.5±140.7               | 436.5±33.2               | 279.5±21.9                           | 13.3±0.0                |
| 20           | 0.50         | 1074.6±18.5                | 239.3±7.0                | 161.6±3.2                            | 12.6±0.1                |
| 22           | 0.50         | 1215.5±37.5                | 287.0±1.4                | 183.7±5.4                            | 13.3±0.3                |
| 24           | 0.50         | 974.7±23.1                 | 225.5±8.7                | 147.1±3.6                            | 13.0±0.2                |

The addition of acid (0.50-0.62 g/100g) resulted in the slight increase in storage modulus ( $G'$ ) at 30 °C, whereas relatively higher increase ( $\cong 1.8$  times) was observed at 90 °C. Addition of sugar (TSS) from 20 to 22 °B increased the  $G'$  and  $G''$  at 30 as well as 90 °C, however, further increase in TSS from 22 to 24 °B resulted in the reduction in the value of  $G'$  and  $G''$ . Dickenso and Merino, [29] also reported the reduction in gel strength by the addition of sugar, they also observed the maximum strength of the gel at 66 g/100mL sugar level, whereas an increase in further sugar percentage (76 g/100 mL) resulted in a reduction of the gel strength. The possible reduction in the gel strength might be due to the partial alteration of pectin gel resulted by the addition of sugar [33]. The viscosity of the fruit based gel like structure is highly dependent on various parameters such as, pectin content, temperature, presence of counter ions and pH [33, 89], the storage modulus ( $G''$ ) and complex viscosity ( $\eta^*$ ) increase until the iso-electric (pH) point of the mango puree (gel like structure) has reached. Manohar, et al., [67] reported the increase in the consistency with the soluble solids of pectinated and depectinated mango pulp. However, beyond the iso-electric pH the values of  $G'$  and  $G''$  reduces. Dickenso and Merino, [29] and Hirashima et al. [37] also reported that for a starch paste there is a pH range where an increase in viscosity can be observed however, the viscosity starts declining at the pH out of the specific range.





(a)

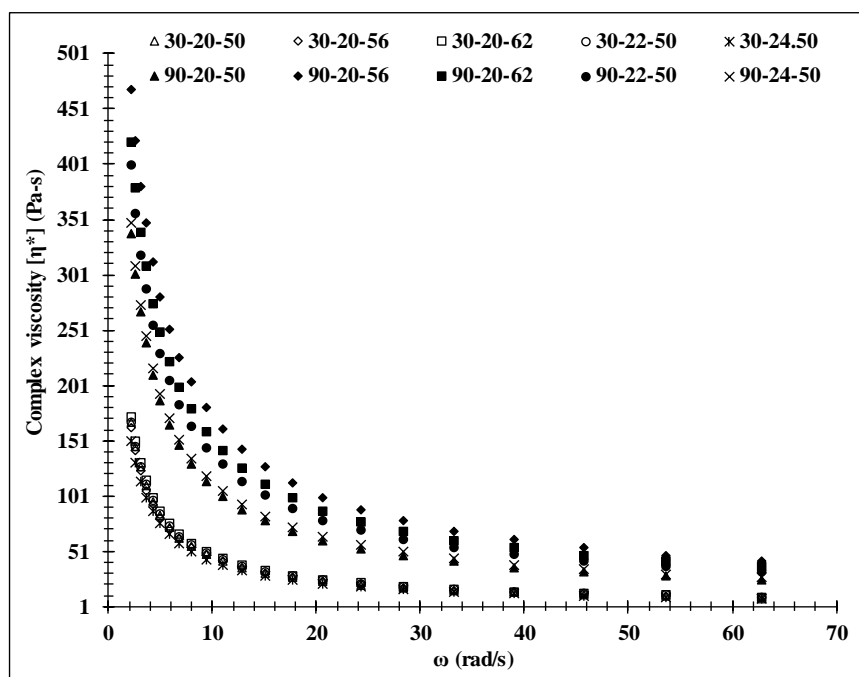


(b)

**Fig. 4.10 Effect of TSS and acid content on dynamic mechanical spectra of mango puree at (a) 30 and (b) 90°C (Legend: storage modulus  $G'$ -(TT-AA) and loss modulus  $G''$ -(TT-AA) where TT represents TSS, and AA represents acid content in fractions)**

In the present experiment, the increase in the acidity from 0.50 to 0.62 g/100g showed an increase in  $G'$ ,  $G''$  and  $\eta^*$ , which indicates the iso-electric pH was yet to achieve. As the addition of acid changes pH, hence the conditions differed. The different conditions result in changing the number of glucose chains due to the leaching of these chains from the starch or other complex molecules. Thus, increase in rheological

parameters could be because of pectin, water and sugar network and entanglement of glucose chains. Guerrero and Alzamora, [33] have also reported the increase in consistency coefficient (Pa.sn) of mango puree when the pH was reduced from 4.2 to 3.0 (or when the acid content was increased). Additionally the gelatinization of starch at a higher temperature (90 °C) may have caused further increase in the  $G'$ ,  $G''$  and  $\eta^*$ , as reported by [37].



**Fig. 4.11 Effect of TSS and acid content on the complex viscosity of mango puree at (a) 30 and (b) 90°C**

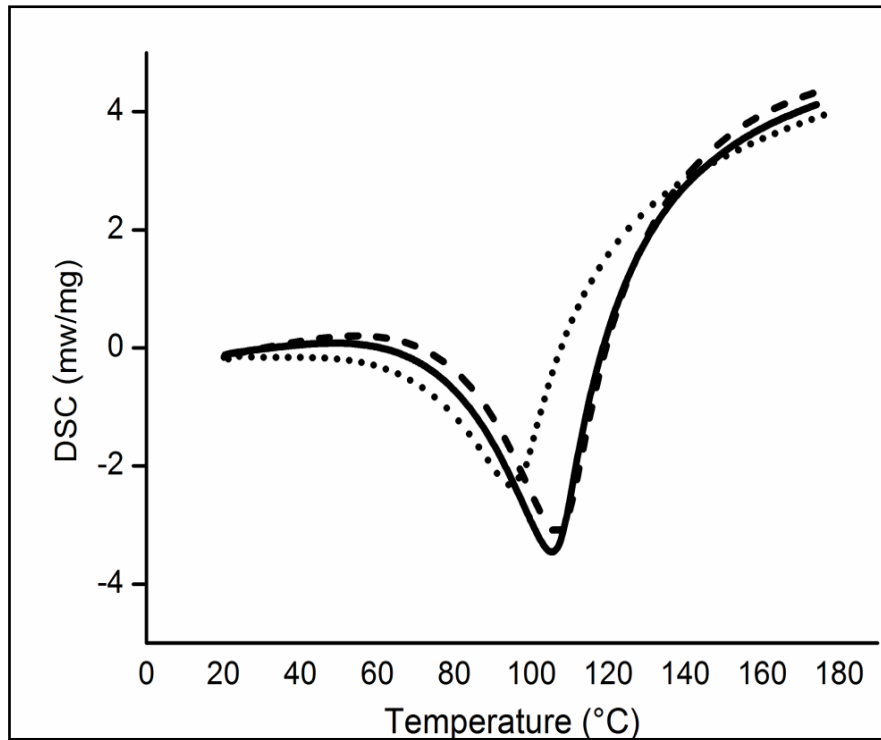
#### 4.2.4 Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA)

In food processing and handling, and equipment design it is important to understand the temperature dependent physical, chemical and bio-chemical changes (such as phase transition, structural modifications in molecules) of mango puree [13]. These thermal properties of fruits and their products get highly influenced by the chemical compositions such as sugar, fats, protein, acid content etc [108]. Additionally, the amount of free and bound water content has an impact on various thermal properties of the material such as specific heat and latent heat of melting [86]. The amorphous sugar may get transformed into crystalline state in the presence of small amount of absorbed moisture [64]. Furthermore the addition of sugar induces the crystallization that would act in the

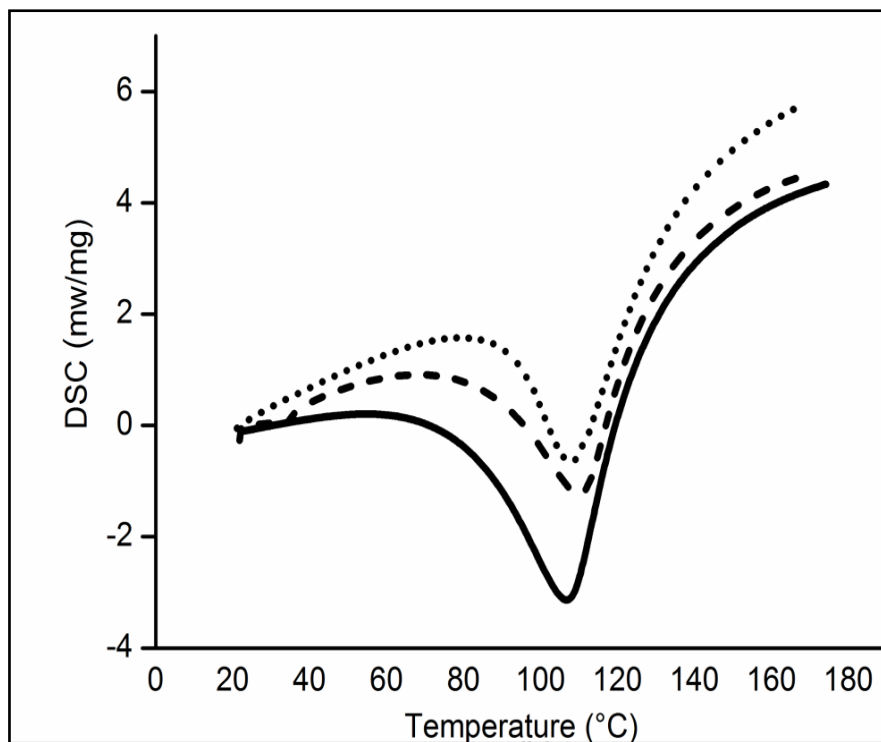
heterogeneous nucleation process [7]. Therefore, present work investigated the effects of acid and TSS content on the thermal behavior of mango puree.

The endotherm scan of the samples was obtained by Differential Scanning Calorimetry (DSC) and presented in Fig. 4.12 (a) and (b). The onset and peak temperatures of phase change in the mango puree were ranged in between 65-70 °C and 95-105°C, respectively, as the acid content increasing and 70-90°C and 105-110°C, respectively, as the TSS increasing from 20 to 24°B. This endothermic phenomenon observed correspond to water loss and fusion [82]. The observed fusion temperatures of sugars do not always correspond to the traditionally accepted melting points. The onset and peak temperatures of the fusion process was found less as compared to sucrose reported by Raemy and Schweizer [82] this was due that impurity in mango puree instead of pure sugar, difference in heating rate, difference in instruments and sample crucibles. The enthalpy of fusion was lower at 24 °B and higher at the lower TSS content, such behavior may be related to the inferring effect of sugar on fusion of other species present or it can be related to the lower mobility of the sugar in the higher TSS content [7]. On the other hand, Fig. 4.13 indicated that an increase in acid (Fig. 4.13a), as well as TSS (Fig. 4.13b), enhanced the thermal stability of mango puree. The mass loss was found to be lower at higher TSS and acid content.

The possible reason for such observations could be molecular mobility and the plasticizing effect of the water with sugar, which has a direct relation with the thermal properties of a material, for example, addition of a biopolymer to a material leads to the increase in its viscosity thus resulting the decrease of diffusional mobility and further changes in thermal properties [87]. Thus, both water activity and the viscosity of the material get affected by changing TSS and acid content. Moraga, et al. [69] studied the relationship of water activity and glass transition of the strawberry tissue and puree and they found a typical linear relationship between these two properties of the food material ( $R^2=0.965$ ). Therefore it can be inferred that the TSS, acid content, water activity, viscosity and thermal properties of any fruit material are related to each other in some way or other.

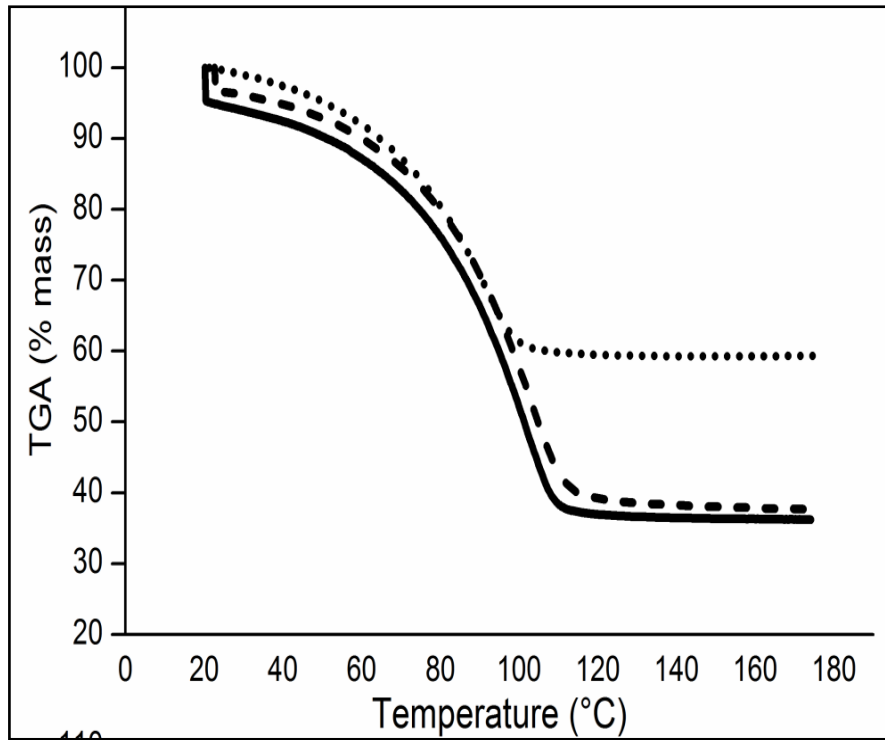


(a)

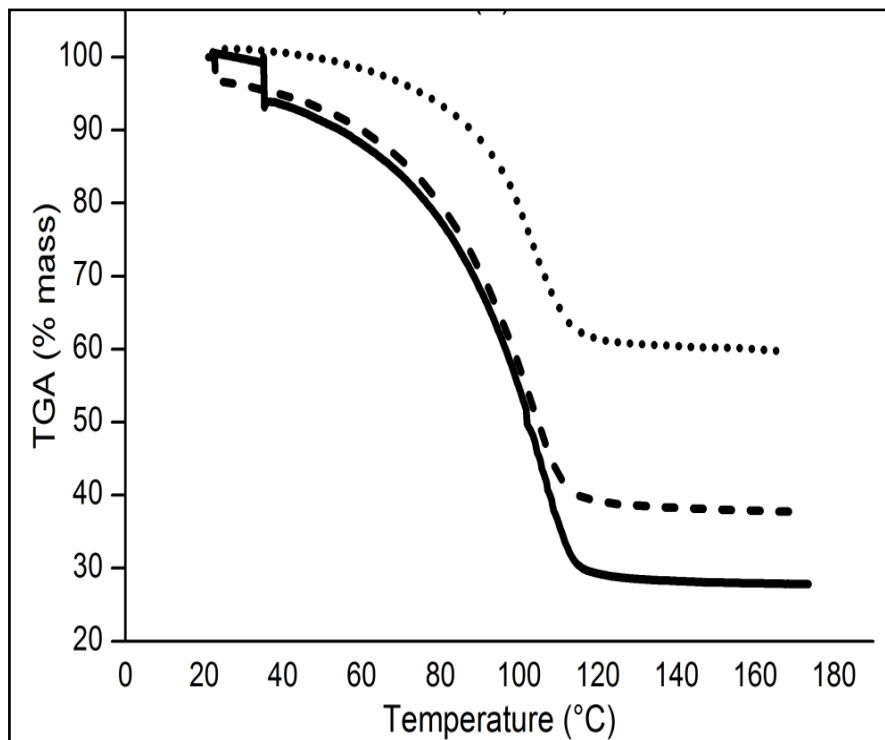


(b)

**Fig. 4.12** DSC plots of mango puree (a) Acid content 0.50 (—), 0.56 (- - -) and 0.62 (.....)%; (b) TSS 20 (—), 22 (- - -) and 24 (.....)°Brix



(a)



(b)

**Fig. 4.13** TGA plots of mango puree (a) Acid content 0.50 (—) 0.56 (- - -) and 0.62 (.....) g/100g; (b) TSS 20 (—) 22 (- - -) and 24 (.....) °Brix

#### 4.2.5 Ohmic Heating (OH) profile of mango puree

During OH of mango puree, the temperature increased linearly Eq. (4.2) with the time of applied Electric Field Strength (EFS). Darvishi, et al. [24]; and Praporscic, et al. [77] also reported the similar heating behavior of pomegranate juice, potato and apple tissue during OH. The increase in EFS caused a rapid increase in the heating rate of the mango puree at all the levels of acid content and TSS (Fig. 4.14 and 4.15), similar results were obtained during the OH of sugarcane juice [95, 96]. Both the acid-modified and TSS modified samples showed the linear change in temperature at all the applied EFS and the regression coefficient ( $R^2$ ) for the linear trend in the change of temperature ranging from 0.982 to 0.997 and 0.930 to 0.997 in samples with different TSS and acid content, respectively.

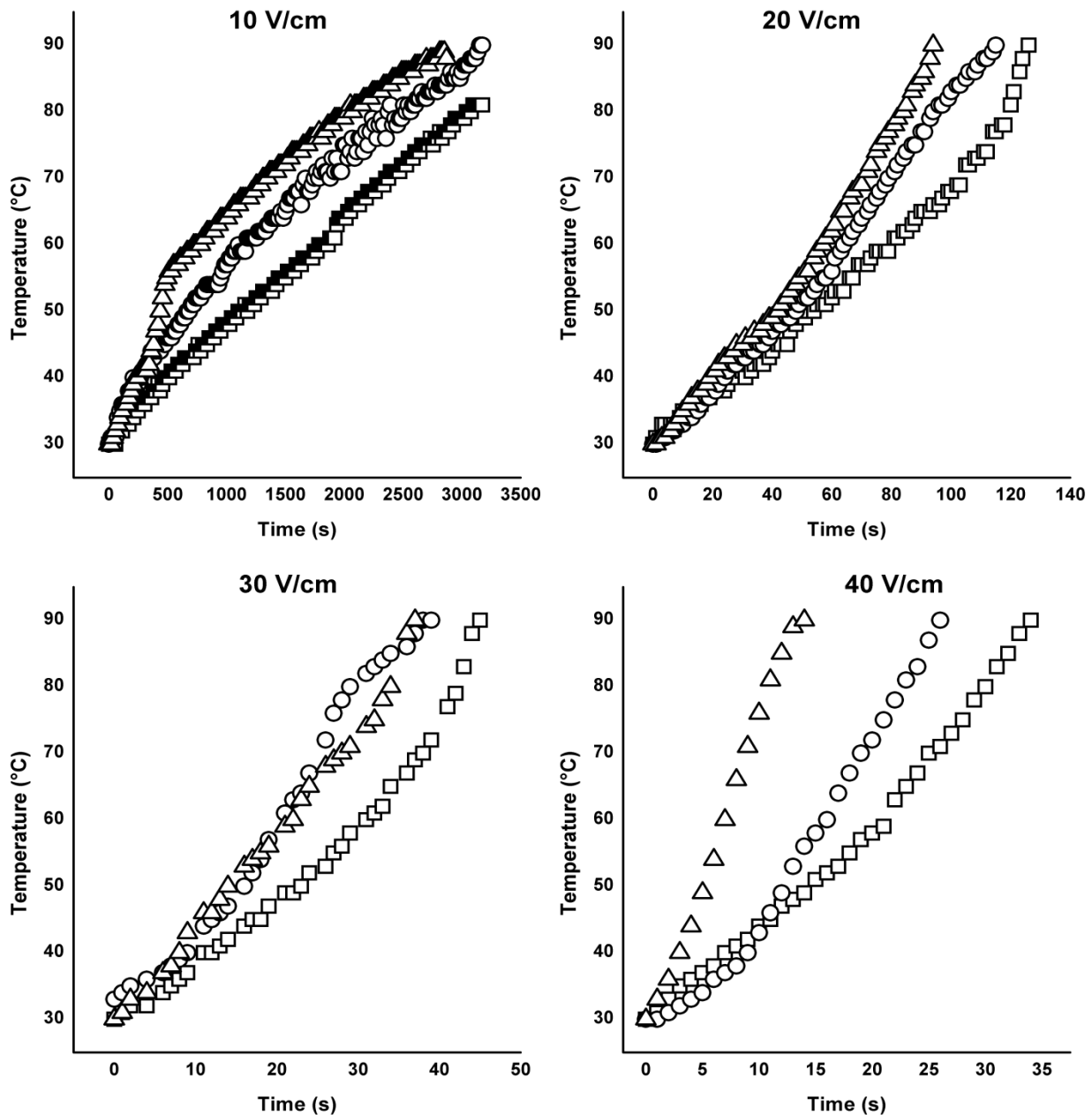
$$\text{Linear model:} \quad T = bt + a \quad \text{Eq. (4.2)}$$

Where, 'b' is slope and 'a' is intercept, 'T' and 't' is temperature ( $^{\circ}\text{C}$ ) and time (min) respectively

##### 4.2.5.1 Effect of acid content on the Ohmic Heating (OH) behavior of mango puree

The increase in acidity from 0.50 to 0.62 g/100g was found to affect the rate of OH in the mango puree. The time required to achieve a temperature of 90  $^{\circ}\text{C}$  in mango puree having an acid content of 0.50 to 0.62 g/100g ranged in between 14 $\pm$ 1 s at 40 V/cm to 64.7 $\pm$ 1.2 min at 10 V/cm. The maximum difference in the rate of heating was found to be at higher EFS than that of lower EFS comparatively as shown in Fig. 4.16. No significant effect of acid content was observed in the heating rate at 10 V/cm, the heating rate in the samples containing 0.50 and 0.62 g/100g acid content was calculated to be 0.016 and 0.018  $^{\circ}\text{C/s}$ , respectively. As reported in the literature [98, 100], the heating rate increased with the applied EFS, therefore heating rate at 40 V/cm was found to be 1.71 and 4.75  $^{\circ}\text{C/s}$  in samples containing 0.50 and 0.62 g/100g acid content respectively. Acids play an important role in making the electric current flow within the food material [49]. Hence, the increase in acid content from 0.5 to 0.62 g/100g was found considerable cause increase in the OH rate of mango puree. However, in the present study, no specific trend or relationship was found between OH rate and EFS of mango puree at different acidity levels, possibly due to fewer data points. Therefore, to establish a model for OH rate and EFS in mango puree, it is recommended to conduct further studies for the evaluation and determination of the heating rate kinetics at different acid content and EFS (>10 V/cm) during OH. It is also possible that due to the presence of other crucial components such as charged salt ions, proteins, etc. in different food material may affect the heating behavior of food material with respect to the

change of EFS and/or acid content. Therefore, the effect of various important components such as acids on the OH behavior will be very useful for the designing of process and processing equipment for OH of foods.



**Fig. 4.14** Change in temperature of samples with varying acid content 0.50 ( $\square$ ), 0.56 ( $\circ$ ) and 0.62 g/100g ( $\triangle$ ) during OH at 10 - 40 V/cm

#### 4.2.5.2 Effect of TSS on the Ohmic Heating (OH) behavior of mango puree

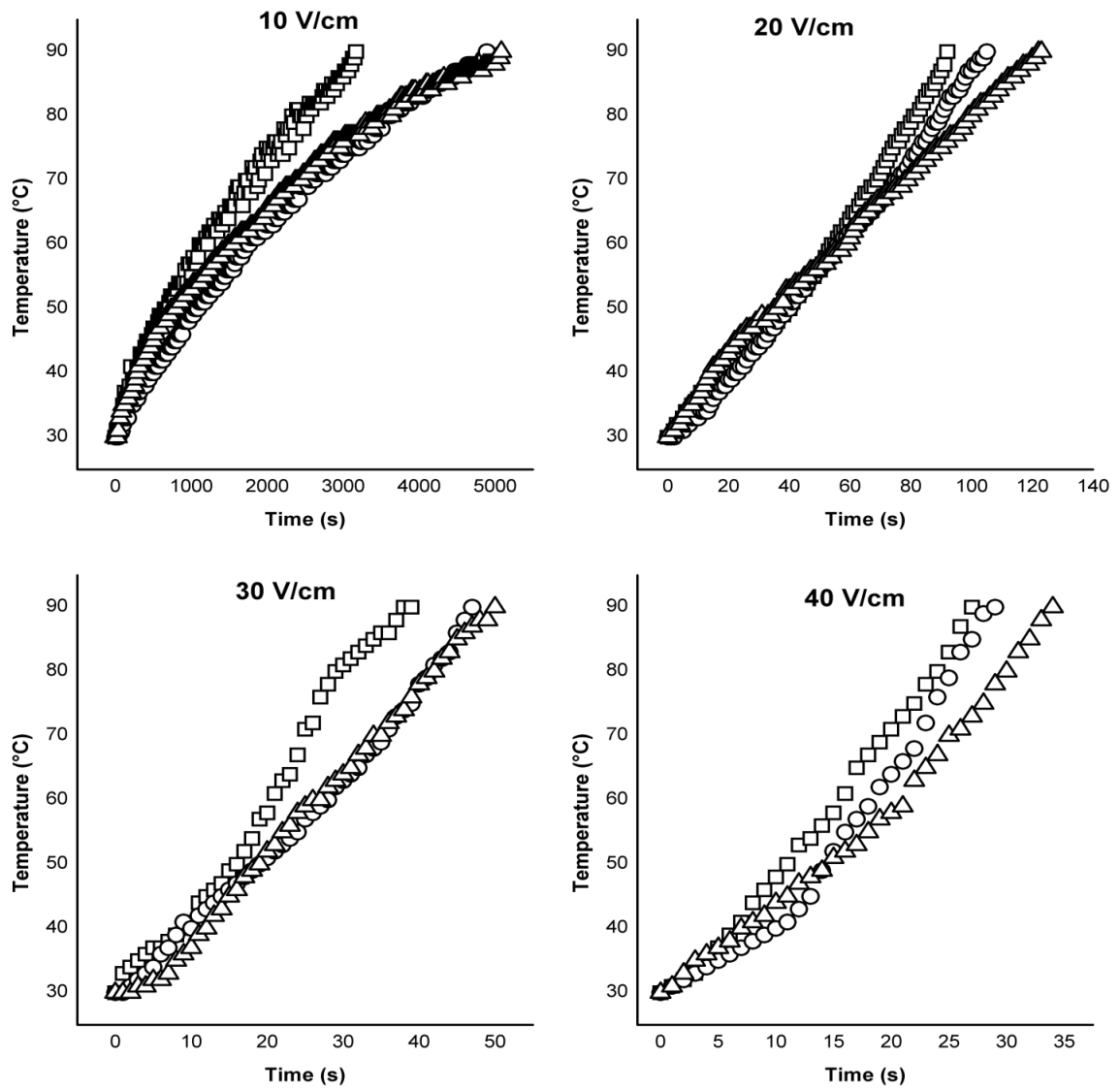
The increase in EFS was found to increase the heating rate in mango puree at all the TSS levels, Icier and Ilicali [41] also reported the similar heating behavior during OH of

apple and sour cherry concentrate. In mango puree, changing TSS from 20-24 °Brix had found to affect the heating behavior or rate during OH using 10 to 40 V/cm EFS. At 10 V/cm the time required to reach the 90 °C was  $52.8 \pm 1.2$ ,  $81.55 \pm 05$  and  $84.7 \pm 0.6$  min at 20, 22 and 24 °B respectively, which was relatively very high than that of heating time at 20 to 40 V/cm. At 40V/cm the time required to reach 90 °C in mango puree having TSS of 20 and 24 °B was observed to be  $28 \pm 1$  and  $36.0 \pm 0.5$  sec respectively, which indicates that the slope of linearity in OH decreased with increasing the TSS of the mango puree at the EFS of 20-40 V/cm. Zareifard et al. [114] has also found that increasing the concentration of the solid content reduces the rate of OH in various food matrixes. Icier and Ilicali [43] reported that the electrical conductivity of orange juice concentrate reduces with increasing the concentration, therefore it can be said that change in the concentration of solids changes the electrical properties of the food material which in turn affects the OH behavior. Hence in conclusion of their study Icier and Ilicali [43] stated that the concentration as one of the critical parameters to be considered for the designing of the OH process and or equipment. Major differences in heating rates were observed at higher EFS when compared with the lower EFS applied for the OH of the mango puree as indicated in Fig. 4.17. Such behavior could be related with the non-polar nature of the sugars, reduction in water activity as well as the increase in the consistency of the material [31], therefore increase in sugar content may cause the hindrances in the OH of fruit material. However, the actual role of sugar and specific sugars in the OH behavior of food material needs to be validated by the further detailed study.

### 4.3 Optimization of Ohmic Heating (OH) process parameters

Optimization of OH parameters was done by RSM using three factorials design as discussed in methodology. The numerical value of responses (dependent variables) viz. Polyphenoloxidase (PPO) activity, Peroxidase (POD) activity, bacterial count and change in color ( $\Delta E$ ) at different levels of independent parameters is listed in Table 4.8 (the results are discussed in detail in the following sections). The effects of ohmic heating treatment with independent variables viz Electric Field Strength (EFS), temperature (T) and time (t) on mango puree are listed as ANOVA results in Table. 4.9. The PPO, POD, bacterial count and change in color ( $\Delta E$ ) were found to follow the quadratic model and the lack-of-fit (LOF) was observed to be insignificant.





**Fig. 4.15** Change in temperature of samples with varying TSS 20(□), 022(○) and 24(△) g/100g during OH at 10 - 40 V/cm

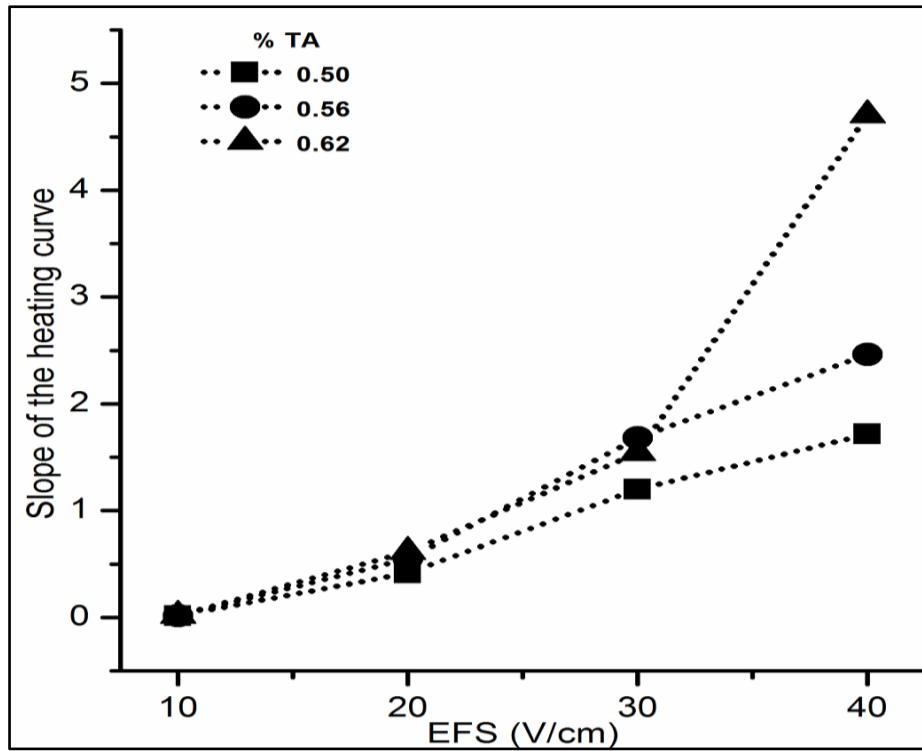


Fig. 4.16 The OH rate at different EFS with different acid content in mango puree

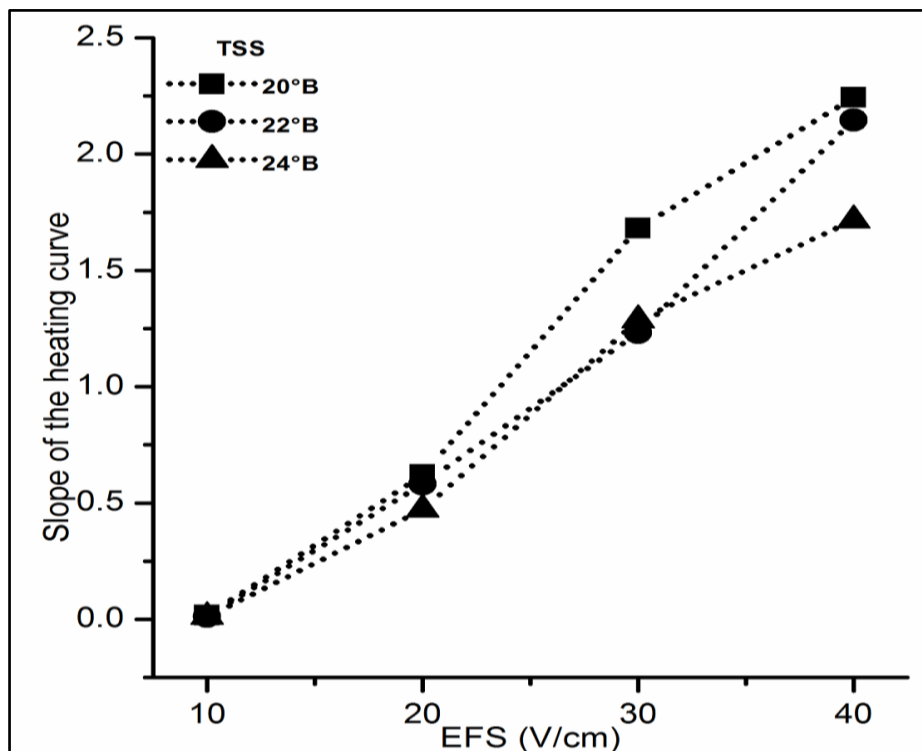


Fig. 4.17 The OH rate at different EFS with different TSS content in mango puree

#### 4.3.1 Effect of Ohmic Heating (OH) on Polyphenoloxidase activity

Polyphenoloxidase (PPO) is an oxidoreductase enzyme mostly responsible for the browning reaction of various fruits and vegetables during cutting, peeling, crushing or juice extraction, etc. The oxidation of o-phenolic compounds to o-quinones in the presence of oxygen causes the browning reaction. Quinones are subsequently polymerized to dark-colored pigments [84, 70]. Therefore, PPO is one of the major challenges in the processing fresh fruits and vegetable products. The OH treatment conditions reduced PPO activity to the range of 11.9 to 42.5 %. However, Abdelmaksoud et al. [1] reported that the residual PPO activity achieved in mango juice during OH was 2.87 %. The higher degree of reduction may be due to the higher applied EFS i.e. 40 V/cm. The residual activity of PPO was affected by treatment conditions are expressed by a quadratic expression as Eq. (4.3). The interaction of time (t) with EFS (E) and temperature (T) was found to have the least effect on the PPO activity; therefore these terms (E.t and T.t) were omitted from the model equation and represented as Eq. (4.4). The square term of time ( $t^2$ ) and interaction of EFS and temperature (ET) were observed to have a positive relationship with the residual PPO activity. The positive effect of ET could be due to the fact that during the heating from ambient to optimal temperature the activity of the enzymes increases. Castro et al. [18] and Saxena et al. [96] also reported a slight increase in the PPO activity during the initial stages of the OH process of PPO extracted from Apple and Sugarcane juice. Such increase in PPO activity during OH could be due to the changing molecular spacing by pulsing of electric current and increasing inter-chain reactions to give a better enzyme-substrate interaction thereof influencing biochemical reactions. The PPO residual activity at the optimized conditions was estimated to be 0.119 (or 11.9 %).

$$\text{PPO (RA)} = 0.2587 - 0.022E - 0.054T - 0.085t + 0.015ET + 3.71 \times 10^{-3}Et + 7.081 \times 10^{-3}Tt - 0.023E^2 - 0.012T^2 + 0.021t^2 \quad [R^2=0.942] \quad \text{Eq. (4.3)}$$

$$\text{PPO (RA)} = 0.2587 - 0.022E - 0.054T - 0.085t + 0.015ET - 0.023E^2 - 0.012T^2 + 0.021t^2 \quad \text{Eq. (4.4)}$$

#### 4.3.2 Effect of Ohmic Heating (OH) on Peroxidase activity

The POD is the most thermally stable enzyme in vegetable systems. As the PPO has more adverse effects on the quality of food material than that of POD, however the resistance of POD to the thermal exposure is found to be higher than any other enzyme present in food material. Therefore, it is generally used as an indicator of the effectiveness of the blanching

process, or if POD is inactivated the other enzymes resulting in quality degradation can be assumed to be inactivated too. However, heating up to the complete inactivation of the POD may result in overprocessing thus, leads to quality degradation and considered more than adequate [2]. The POD inactivation was relatively slower than that of the PPO, due to the higher resistance of POD relatively. POD activity was reduced up to a range of 21.7 to 49.9 % in the present study. The reduction was observed to follow the quadratic model with nonsignificant lack of fit and coefficient of determination ( $R^2$ ) of 0.975; the equation is represented as Eq. (4.5). The model suggests that the ET, Et and  $E^2$  have the least effect on the POD activity. Also, E with a coefficient of -0.013 was found to have a minimum role in POD inactivation as compared to T (-0.059) and t (-0.074). Therefore, the equation was reduced to Eq. (4.6). These results also suggested that the inactivation of the POD in mango puree was mostly due to the thermal damage of the enzyme.

$$\text{POD (RA)} = 0.365 - 0.013E - 0.059T - 0.074t - 8.068 \times 10^{-3}ET + 8.238 \times 10^{-4}Et + 0.013Tt + 1.853 \times 10^{-3}E^2 - 0.027T^2 + 1.606 \times 10^{-3}t^2 \quad [R^2 = 0.967] \quad \text{Eq.(4.5)}$$

$$\text{POD (RA)} = 0.365 - 0.013E - 0.059T - 0.074t + 0.013Tt - 0.027T^2 \quad \text{Eq.(4.6)}$$

The comparatively higher coefficient of EFS of PPO than that of POD (Eq. (4.4) and (4.6)) should be due to the nonthermal effects of OH on PPO. The PPO contains Copper as a metallic prosthetic group and the applied electric field results in the removal of the metal from enzyme hence cause additional inactivation of enzyme activity as compared to that of solely due to thermal damages. Whereas there is no metallic prosthetic group in POD, therefore, the inactivation was observed mostly due to thermal effects (time and temperature) of OH. The presence of an electric field during OH reduced the time of PPO inactivation. Therefore, ohmic and conventional heating resulted in significantly different thermal resistance constants (z-values) of PPO inactivation in apple cubes [18]. Similar observations were also reported by Saxena et al. [96] in sugarcane juice, Makroo et al. [66] in watermelon juice and Icier et al. [45] in grape juice. Castro et al. [18] suggested that, by OH similar PPO inactivation can be achieved with the lower thermal destruction of vitamins and pigments and fruit texture, thus increasing the final quality of the products as compared to conventional thermal processing.

#### 4.3.3 Effect of Ohmic Heating (OH) on Bacterial count

The bacterial count is one of the fundamental and most commonly used methods to evaluate the microbial stability of food material. The effect of OH parameters on microbial

inactivation was studied in terms of reduction in bacterial count estimated by nutrient agar method. The model equation obtained is listed as Eq. (4.7). The effect of the interaction of independent variables and quadratic terms was found to be very small. Therefore, these terms were removed in the model. The final equation obtained regarding the dependency of microbial reduction is mentioned as Eq. (4.8).

**Table 4.8 Response values at different experimental conditions**

| Run | EFS<br>(V/cm) | Temperature<br>(°C) | Time<br>(min) | Residual activity |       | $\Delta E$ | Bacterial count<br>(% reduction) |
|-----|---------------|---------------------|---------------|-------------------|-------|------------|----------------------------------|
|     |               |                     |               | PPO               | POD   |            |                                  |
| 1   | 15            | 85                  | 1             | 0.425             | 0.499 | 1.978      | 0.075                            |
| 2   | 15            | 90                  | 1.5           | 0.239             | 0.369 | 1.999      | 0.048                            |
| 3   | 20            | 85                  | 2             | 0.218             | 0.313 | 3.160      | 0.022                            |
| 4   | 15            | 95                  | 1.5           | 0.171             | 0.314 | 2.151      | 0.028                            |
| 5   | 15            | 85                  | 2             | 0.246             | 0.328 | 2.828      | 0.021                            |
| 6   | 20            | 90                  | 1.5           | 0.263             | 0.368 | 2.394      | 0.022                            |
| 7   | 25            | 90                  | 2             | 0.150             | 0.275 | 3.060      | 0.005                            |
| 8   | 25            | 90                  | 1.5           | 0.187             | 0.353 | 2.854      | 0.017                            |
| 9   | 25            | 85                  | 1             | 0.330             | 0.476 | 1.677      | 0.043                            |
| 10  | 25            | 90                  | 1             | 0.342             | 0.464 | 2.194      | 0.036                            |
| 11  | 25            | 85                  | 1.5           | 0.263             | 0.397 | 2.042      | 0.019                            |
| 12  | 20            | 90                  | 1.5           | 0.259             | 0.362 | 2.341      | 0.027                            |
| 13  | 15            | 85                  | 1.5           | 0.317             | 0.398 | 2.325      | 0.050                            |
| 14  | 25            | 95                  | 1.5           | 0.133             | 0.243 | 2.992      | 0.010                            |
| 15  | 20            | 90                  | 1.5           | 0.257             | 0.367 | 2.530      | 0.029                            |
| 16  | 25            | 95                  | 2             | 0.119             | 0.217 | 3.061      | 0.004                            |
| 17  | 20            | 95                  | 1             | 0.286             | 0.341 | 2.593      | 0.048                            |
| 18  | 20            | 95                  | 2             | 0.141             | 0.225 | 3.059      | 0.011                            |
| 19  | 20            | 95                  | 1.5           | 0.221             | 0.296 | 2.717      | 0.014                            |
| 20  | 20            | 90                  | 1.5           | 0.278             | 0.350 | 2.494      | 0.025                            |
| 21  | 20            | 85                  | 1.5           | 0.315             | 0.402 | 2.431      | 0.033                            |
| 22  | 25            | 85                  | 2             | 0.179             | 0.304 | 2.588      | 0.010                            |
| 23  | 20            | 85                  | 1             | 0.397             | 0.482 | 2.200      | 0.055                            |
| 24  | 15            | 95                  | 2             | 0.122             | 0.224 | 2.487      | 0.012                            |
| 25  | 15            | 90                  | 1             | 0.409             | 0.457 | 1.696      | 0.071                            |
| 26  | 15            | 90                  | 2             | 0.187             | 0.317 | 2.540      | 0.018                            |
| 27  | 25            | 95                  | 1             | 0.266             | 0.302 | 2.254      | 0.024                            |
| 28  | 20            | 90                  | 2             | 0.176             | 0.276 | 2.874      | 0.015                            |
| 29  | 20            | 90                  | 1             | 0.368             | 0.434 | 2.355      | 0.056                            |
| 30  | 20            | 90                  | 1.5           | 0.244             | 0.375 | 2.310      | 0.025                            |
| 31  | 20            | 90                  | 1.5           | 0.254             | 0.369 | 2.484      | 0.027                            |
| 32  | 15            | 95                  | 1             | 0.254             | 0.369 | 1.828      | 0.059                            |

The reduction in microbial load was strongly dependent on temperature; however, it is visible from the equation that the EFS also play some role in microbial reduction. These

observations imply that there are some nonthermal effects of OH on microbial reduction; Rodrigues et al. [85] compared the effect of OH and conventional heating on *Staphylococcus aureus* in infant formula. It was found that the D-values reduced with the temperature of conventional and OH thermal processing, however, significant differences were found in D-values of the OH (0.53 min) and conventional (1.42 min) heating at 65 °C. Saxena et al. [95] also reported additional nonthermal effects of OH on the reduction of *L. mesenteroides* in sugarcane juice as compared to that of conventional heating. These results were also support by the obtained Eq. (4.8) which suggests that EFS has some additional role in microbial reduction. However, on the contrary Bastias et al. [10] reported that there is no difference between ohmic and conventional heating regarding the inactivation of microbes while studying Chilean blue mussels, these results may be the outcome of using lower EFS i.e. 9.15 V/cm for the OH process.

**Table 4.9 ANOVA results of the suggested model**

| Source         | PPO     |                                 | POD     |                                 | Plate Count |                                 | ΔE      |                                 |
|----------------|---------|---------------------------------|---------|---------------------------------|-------------|---------------------------------|---------|---------------------------------|
|                | F-Value | P-Value<br>( $\times 10^{-3}$ ) | F-Value | P-Value<br>( $\times 10^{-3}$ ) | F-Value     | P-Value<br>( $\times 10^{-3}$ ) | F-Value | P-Value<br>( $\times 10^{-3}$ ) |
| EFS (E)        | 57.54   | < 0.10                          | 102.63  | < 0.10                          | 82.43       | < 0.10                          | 16.93   | < 0.10                          |
| Temp. (T)      | 22.67   | < 0.10                          | 17.37   | 0.40                            | 176.61      | < 0.10                          | 16.20   | 0.60                            |
| Time (t)       | 134.25  | < 0.1                           | 333.25  | < 0.10                          | 52.93       | < 0.10                          | 7.11    | 14.10                           |
| ExT            | 332.35  | < 0.10                          | 528.18  | < 0.10                          | 461.92      | < 0.10                          | 91.96   | < 0.10                          |
| Ext            | 7.29    | 13.10                           | 4.10    | 55.10                           | 0.86        | 4.20                            | 20.67   | 0.20                            |
| Txt            | 0.42    | 524.30                          | 0.04    | 838.00                          | 27.89       | < 0.10                          | 0.15    | 698.30                          |
| E <sup>2</sup> | 1.52    | 230.90                          | 11.90   | 2.30                            | 1.20        | 285.30                          | 1.81    | 192.70                          |
| T <sup>2</sup> | 10.11   | 4.30                            | 0.13    | 722.80                          | 0.02        | 870.10                          | 14.03   | 1.10                            |
| t <sup>2</sup> | 3.04    | 95.3                            | 27.55   | < 0.10                          | 1.41        | 246.90                          | 1.09    | 308.50                          |
| LOF            | 8.72    | 7.3                             | 0.09    | 758.40                          | 19.38       | 0.20                            | 1.44    | 242.40                          |
| R <sup>2</sup> | 3.76    | 74.30                           | 3.17    | 103.10                          | 3.18        | 102.70                          | 4.28    | 57.70                           |
|                | 0.959   |                                 | 0.976   |                                 | 0.873       |                                 | 0.971   |                                 |

$$\begin{aligned} \text{Bacterial count (\% reduction)} = & 0.027 - 0.011E - 6.556T - \\ & 0.019t + 1.022 \times 10^{-3}ET + 5.828 \times 10^{-3}Et + 1.208 \times 10^{-3}Tt + \\ & 2.365 \times 10^{-4}E^2 - 1.700 \times 10^{-3}T^2 + 6.293 \times 10^{-3}t^2 \quad [R^2 = 0.959] \end{aligned} \quad \text{Eq. (4.7)}$$

$$\text{Plate count (\% reduction)} = 0.027 - 0.011E - 6.556T - 0.019t \quad \text{Eq. (4.8)}$$

#### 4.3.4 Effect of Ohmic Heating (OH) on the change in color (ΔE)

The color of the mango and its products is a very critical quality parameter and processing causes serious changes in it. Ndiaye et al. [72] and Kaushik et al. [50] studied the effect of steam blanching and high-pressure processing on the color of mango slices and pulp

respectively. The  $\Delta E$  increased with the holding time at different HPP conditions (100-600 MPa) whereas, in steam blanching of mango slices the color change stopped after 3 min of the treatment, probably due to the inactivation of color degrading enzymes such as PPO. In the present study, the most complex expression of dependency of the dependent parameter on the independent parameter was for  $\Delta E$  (Eq. (4.9)), as visible from the surface plot Fig. 4.18 (d). The  $\Delta E$  was found to be depended on all the terms of the quadratic model, with a determination coefficient ( $R^2$ ) of 0.972 and insignificant lack of fit ( $p < 0.01$ ).

The time of OH process was found to have the highest coefficient thus effectiveness on the color change. Mercali et al. [68] also reported that in acerola pulp the value of  $\Delta E$  increases with OH time and it was following the first order trend, they also found that higher color changes occur at a lower frequency (10 Hz) to that of higher frequency (100 KHz) of electric current. However, Bhat et al. [11] reported that OH helps in color retention to a higher level as compared to conventional heating of bottle guard juice, possibly due to higher damage to the color degrading enzymes. The moderate heat treatment sometimes enhances the color of the vegetable product by releasing of color compounds from the matrix of the vegetable tissue network.

$$\Delta E = 2.506 + 0.160E + 0.106T + 0.382t + 0.222ET + 0.019Et - 0.065Tt - \quad \text{Eq. (4.9)}$$

$$0.236E^2 + 0.065T^2 + 0.075t^2 \quad [R^2 = 0.822]$$

#### 4.3.5 Optimized condition and Desirability

The constraints of the optimization process for the selected design are tabulated in Table 4.10. The top ten optimized conditions suggested by the RSM are listed in Table 4.11. Although the most desirable condition suggested by the RSM design was OH at 95 °C for 1.942 min using EFS 15 V/cm. However, after analyzing the desirability and the process parameters of the first and seventh suggested conditions, it was found that the time of treatment can be reduced by 9.25 % at the compromise 0.132 % reduction in desirability between the two conditions (Table 4.11). As listed in Table 4.12 the PPO, POD and microbial activity was higher by just 2.7, 1.7 % and 0.001 % respectively. However, lesser color change was at the seventh suggested condition as compared to the first one. Hence the seventh suggested condition was considered and selected as the optimized condition, with the desirability of 0.794 and the processing conditions were heating at 95 °C for 1.91 min using 15 V/cm EFS. The overall desirability and desirability of the individual parameter is shown in Fig. 4.19.

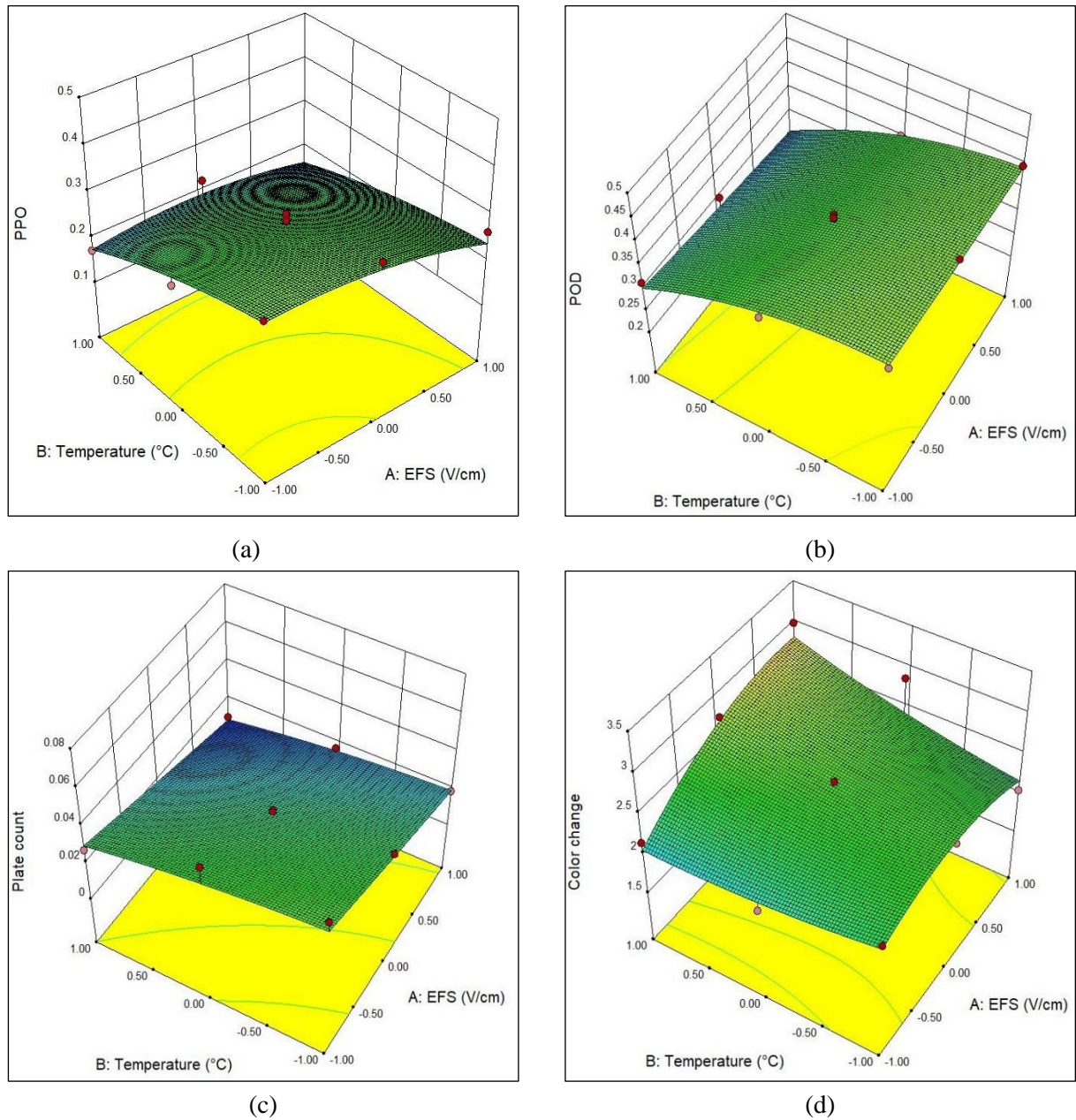


Fig. 4.18 Surface plots of the (a) PPO; (b) POD; (c) Plate count; and (d)  $\Delta E$

Table 4.10 Constraints of the optimization process

| Parameter   | Name             | Goal     | Lower Limit | Upper Limit | Optimized condition |
|-------------|------------------|----------|-------------|-------------|---------------------|
| Independent | EFS (V/cm)       | in range | 15          | 25          | 15                  |
|             | Temperature (°C) | in range | 85          | 95          | 95                  |
|             | Time (min)       | in range | 1           | 2           | 1.91                |
| Dependent   | PPO              | minimize | 0.119       | 0.424       | 0.122               |
|             | POD              | minimize | 0.216       | 0.499       | 0.253               |
|             | $\Delta E$       | minimize | 1.676       | 3.159       | 2.350               |
|             | Bacterial count  | minimize | 0.003       | 0.074       | 0.015               |



**Table 4.11 The ten best conditions suggested by the RSM**

| Number   | EFS          | Temperature | Time         | PPO          | POD          | $\Delta E$   | BC           | Desirability |                 |
|----------|--------------|-------------|--------------|--------------|--------------|--------------|--------------|--------------|-----------------|
| 1        | -1.00        | 1.00        | 0.884        | 0.119        | 0.249        | 2.382        | 0.014        | 0.795        |                 |
| 2        | -1.00        | 1.00        | 0.884        | 0.119        | 0.249        | 2.382        | 0.014        | 0.795        |                 |
| 3        | -1.00        | 1.00        | 0.872        | 0.120        | 0.250        | 2.376        | 0.014        | 0.794        |                 |
| 4        | -1.00        | 1.00        | 0.848        | 0.121        | 0.251        | 2.366        | 0.014        | 0.794        |                 |
| 5        | -1.00        | 1.00        | 0.829        | 0.122        | 0.252        | 2.358        | 0.015        | 0.794        |                 |
| 6        | -1.00        | 1.00        | 0.921        | 0.118        | 0.247        | 2.398        | 0.013        | 0.794        |                 |
| <b>7</b> | <b>-1.00</b> | <b>1.00</b> | <b>0.809</b> | <b>0.122</b> | <b>0.253</b> | <b>2.350</b> | <b>0.015</b> | <b>0.794</b> | <b>Selected</b> |
| 8        | -0.99        | 1.00        | 0.892        | 0.119        | 0.248        | 2.393        | 0.014        | 0.793        |                 |

**Table 4.12 Difference between the 1<sup>st</sup> and 7<sup>th</sup> suggested optimization condition**

| Number   | EFS   | Temperature | Time  | PPO   | POD   | Color change | Bacterial count | Desirability |
|----------|-------|-------------|-------|-------|-------|--------------|-----------------|--------------|
| 1        | -1.00 | 1.000       | 0.884 | 0.119 | 0.249 | 2.382        | 0.014           | 0.795        |
| 7        | -1.00 | 1.000       | 0.809 | 0.122 | 0.253 | 2.350        | 0.015           | 0.794        |
| % change | -     | -           | 9.246 | 2.747 | 1.744 | 1.356        | 6.750           | 0.132        |

#### 4.3.6 Validation

The validation of the data was carried out by checking the experimental and predicted value in the optimization condition at a significance level of 95 %. The results were found to be within the confidence range. The results of validation are shown in Table 4.13.

**Table 4.13 Experimental and predicted values of PPO, POD residual activity, bacterial count and color change at optimized condition**

| Parameter            | Unit              | Predicted value     | Experimental value  | % deviation |
|----------------------|-------------------|---------------------|---------------------|-------------|
| PPO                  | Residual activity | 0.122 <sup>a</sup>  | 0.121 <sup>a</sup>  | 0.820       |
| POD                  | Residual activity | 0.253 <sup>a</sup>  | 0.248 <sup>a</sup>  | 1.976       |
| Color ( $\Delta E$ ) | --                | 2.350 <sup>a</sup>  | 2.358 <sup>a</sup>  | 0.340       |
| Bacterial count      | % CFU reduction   | 0.0137 <sup>a</sup> | 0.0132 <sup>a</sup> | 3.650       |

<sup>x</sup> values in the same alphabet were significantly not different at ( $p < 0.05$ )

Further, in the present study, the mango puree was treated by the OH optimized condition and compared with the conventional heating method (hot water). The effects of come up time and holding time on various important parameters were studied and compared. Finally, the treated samples were stored for three months (90 days) under ambient conditions. The details are discussed in the follow-up section.

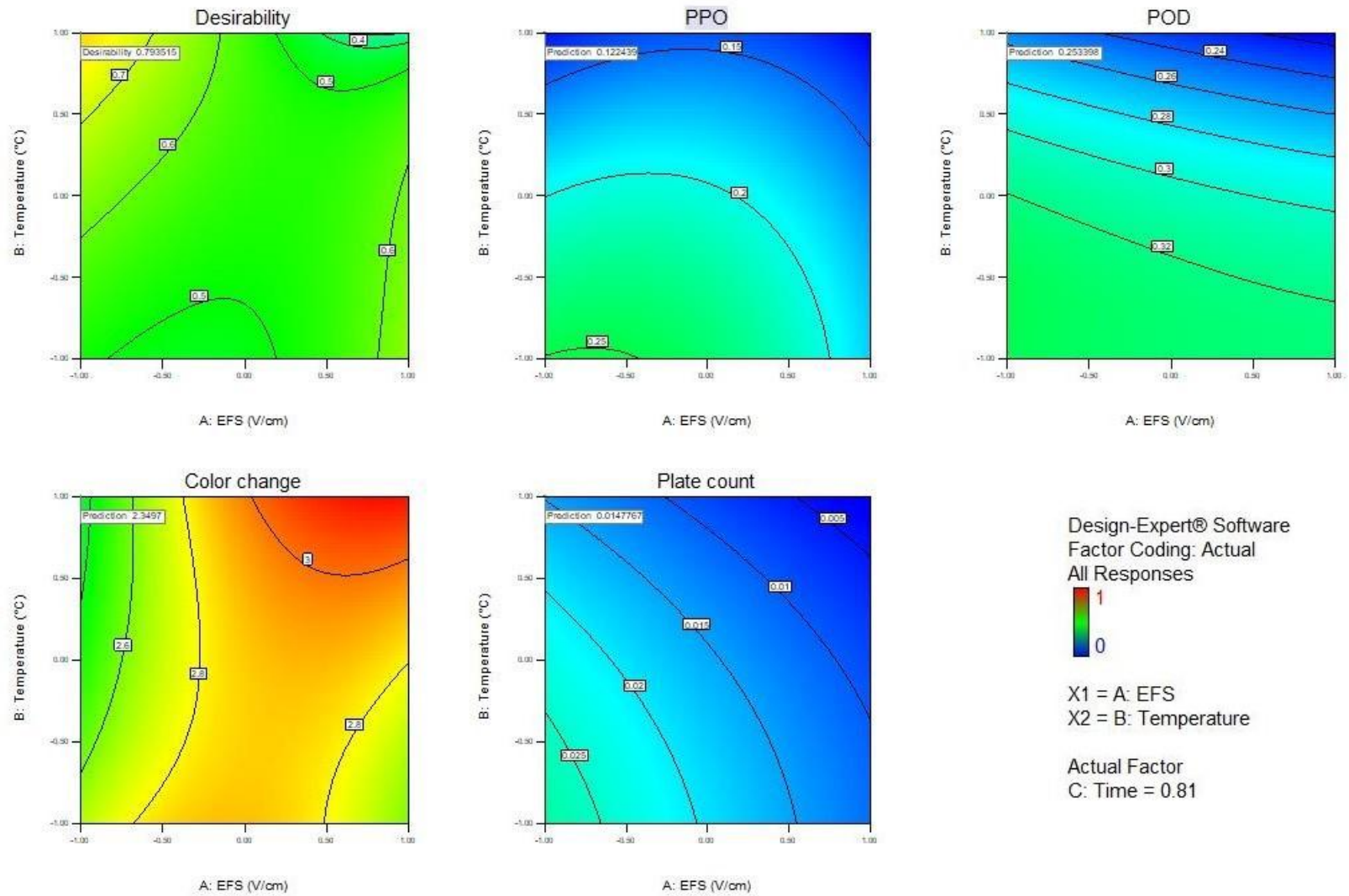


Fig. 4.19 Desirability of the optimized condition

#### **4.4 Kinetics of Enzyme, microbial inactivation and color change during OH**

The action of enzymes and microbial contamination are the major reason for fruits and vegetable spoilage. Polyphenoloxidase (PPO) and Peroxidase (POD) are two major enzymes naturally present in mango fruit. They encourage the objectionable changes in the quality characteristics of mango puree such as color, flavor and nutritional composition. These enzymes differ in their chemical structure hence have different activity and effectiveness. POD is reported to have the highest thermal resistance among all the naturally occurring enzymes in fruits and vegetables. Therefore, it is used as an end point indicator for processing methods applied aiming the inactivation of enzymes such as blanching [47]. The PPO is an oxidoreductase, responsible for the undesirable enzymatic browning of fruits and vegetables. Microbial and parasitic contamination may occur at any stage of food production or post-harvest handling viz processing, storage or transportation etc. These biological agents could be pathogenic or biological hazard to humans and animals and poses health risks, few may be so much resistant to the environment that they can even survive during preservation treatments. Such food may carry some risk of microbial contamination if not handled properly prior to its consumption. Therefore, microbial safety is considered one of the most important objectives for shelf life extension and food safety [71].

The color of the food products especially mango puree is an important quality attribute and indicator of nutritional wholesomeness of the product. The change in color of mango puree may be due to many reasons such as enzymatic reactions, physical damage such as over-heating, pH change, oxidation, duration and temperature of storage etc. Therefore, the maintenance of the color of mango puree during processing and storage is also considered to be major challenge. It is important for the fruit processors that the product retains maximum of its natural bright and attractive color [4]. Although the color change kinetics during processing of food material is a complex phenomenon. Ahmed et al. [4] studied the color degradation kinetics during thermal processing of mango puree.

Kinetic study is important for the processing design and formulation as it give very useful information regarding the quality change occurring during the processing. However, limited literature is available on kinetic study of change in PPO, POD, color and microbial reduction during OH of mango puree. Therefore, the aim of the follow up

section was to study the kinetics of PPO, POD, microbial inactivation and color change in mango puree during OH.

#### 4.4.1 Enzyme inactivation

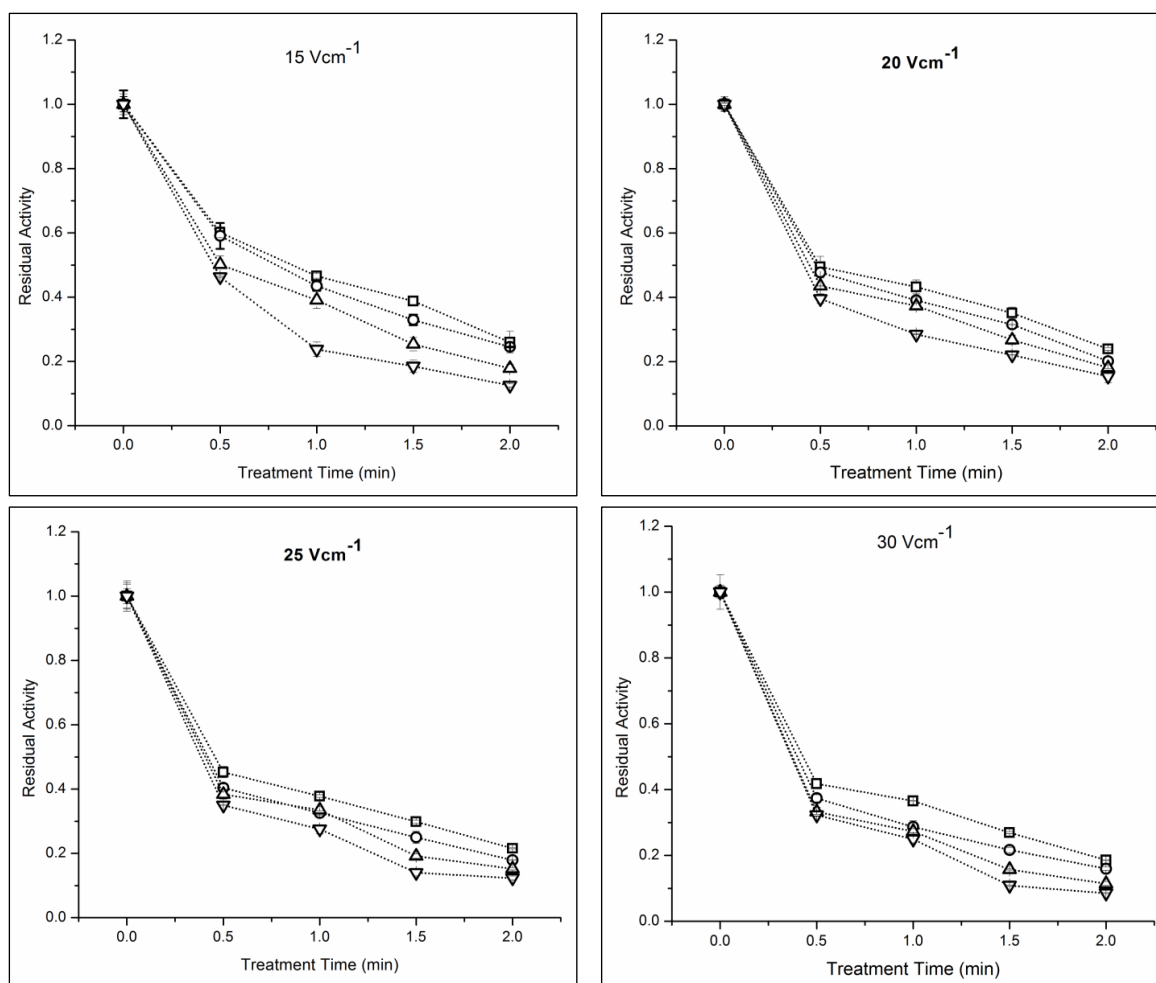
Generally it is adopted that enzyme inactivation follows first order pattern, which is based on the assumption that the break of a single bond or a structure is sufficient to inactivate the enzyme. However, it is known that the inactivation process is much more complex [17], precisely the inactivation by OH is reported to be more complex [18]. Thermal denaturation of enzymes happens because of relocation and or destruction of non-covalent bonds such as hydrogen bonds, hydrophobic interactions, and ionic bonds of the tertiary protein structure. However during OH the presence of electric field effects the biochemical reactions by altering molecular spacing and increasing inter-chain reactions [18]. Therefore, under the similar thermal histories between OH and conventional heating the enzyme inactivation may not be similar.

##### 4.4.1.1 Polyphenol oxidase (PPO) inactivation

The PPO activity in the fresh mango pulp was found to be  $162 \pm 4.8$  units. The PPO inactivation plots at different EFS are shown in Fig. 4.20. The residual activity reduced with the time and at higher temperature the rate of inactivation was higher. The higher inactivation could be due to speedy destruction of non-covalent bonds. As evident from the plots (Fig. 4.20) that the maximum reduction rate of enzyme inactivation was achieved during the early heating period i.e. up to 0.5 min at every EFS – Temperature combination followed by lower reduction rate resembles the exponential reduction in enzyme activity. The residual activity of PPO enzymes was fitted to various kinetic models reported in literature and listed as Eq. (3.15 – 3.20).

The PPO inactivation was best fitting with the Distinct isozyme model (Eq. 3.16). The model was selected on the basis of least chi-square ( $\chi^2$ ) and maximum  $R^2$  value as shown in Table 4.14. The closeness in best fit can also be seen in Fig. 4.21(a) by the plot of experimental and predicted value. The data points are falling within 5% confidence interval. Brochier et al. [17] have also reported similar findings during studies of enzyme inactivation in sugarcane juice by OH. Distinct isozyme model suggests that the enzyme inactivation occurs by the sum of two exponential decays, one is thermo-labile (rapid inactivation) and the other is thermo-resistant (slow inactivation) [112]. The same behavior is quite visible from the plots the rapid initial reduction shows the thermo labile decay whereas the consequent slow inactivation was due to the thermo resistant decay of

the PPO activity in mango puree during OH. Saxena et al. [94] have also reported that the PPO inactivation follows the Distinct isozyme model during OH of sugarcane juice however their study also reported that the PPO inactivation by conventional hot water heating followed the  $n^{\text{th}}$  order inactivation kinetics. The differences in the inactivation patten could be due to the presence of the electric field or because of the additional effects of the electric current generated during the OH process.

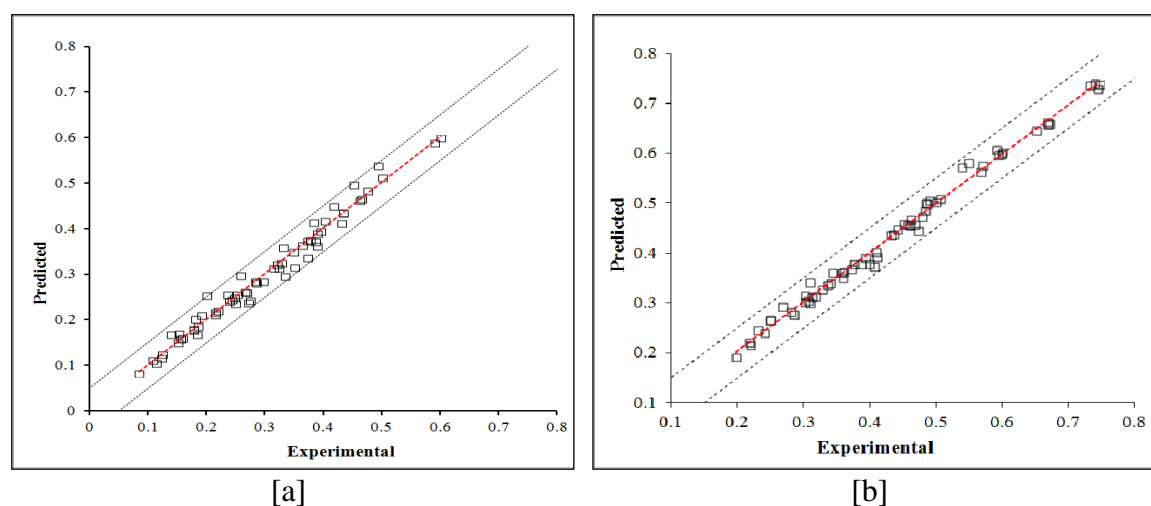


**Fig. 4.20** The PPO inactivation (residual activity) during OH at different EFS and temperature (°C) 80 (□), 85 (○), 90 (△) and 95 (▽)

The enzyme activity has been reported to increase in the temperature range 20-60 °C during OH, Icier et al. [45] observed increase in POD of grape juice and similar increase in the PPO activity was reported by Brochier et al. [17] in sugarcane juice, however present study didn't witness any such increase in the PPO activity due to the higher treatment temperature range i.e. 80-95 °C. The kinetic and statistical parameters of the distinct isozyme model of PPO in mango puree are presented in Table 4.15.

**Table 4.14 Chi square and R<sup>2</sup> values of selected models for the PPO inactivation**

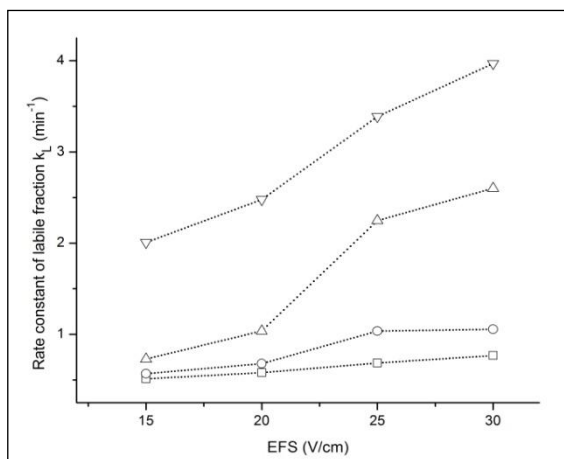
| S No | Model                    | $\chi^2$                  | R <sup>2</sup>      |
|------|--------------------------|---------------------------|---------------------|
| 1    | First order              | 6.285-1092.40             | 0.407-0.997         |
| 2    | <b>Distinct isozymes</b> | <b>0.0000048- 0.00218</b> | <b>0.978 -0.999</b> |
| 3    | Two-fraction             | 0.024-2183.648            | 0.222-0.999         |
| 4    | Fractional conversion    | 0.590- 54.453             | 0.818-0.999         |
| 5    | Weibull distribution     | 0.130-26.90               | 0.926-0.999         |
| 6    | n <sup>th</sup> order    | 0.409-36.807              | 0.902-0.999         |

**Fig. 4.21 The Experimental and predicted values of bested fitted model for PPO [a] and POD [b] inactivation**

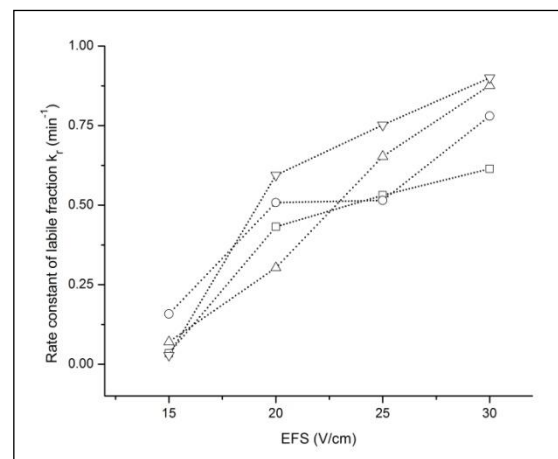
The distinct enzyme behavior illustrate that the enzyme inactivation occurs by the sum of two exponential decays. One will be heat labile that is sensitive to heat, thus rapid inactivation will occur in this fraction whereas the other one will be thermo labile or resistant to heat relatively [20]. The EFS was observed have least effect on  $k_1$  at lower temperature (80 °C), however as the temperature was increased the effect of EFS was found to increase sharply while increasing the EFS from 15 to 30 V/cm. The observations are depicted in Fig. 4.22. The rate constant of resistant fraction ( $k_r$ ) was observed to increase with EFS; however, it was not following any specific trend with the temperature Fig. 4.23. Therefore, further studies are recommended to evaluate the effect of temperature on the rate constant of PPO enzyme inactivation during OH of mango puree.

The effect of EFS on the enzyme inactivation is evident from the values of Decimal reduction time (D-value) and Inactivation resistance constant (z-value) of the two fraction of PPO as shown in Table 4.16. The  $D_1$  was observed to reduce by 74.38, 76.57, 79.76 and 80.66 % when the temperature was increased from 80 to 95 °C at 15, 20, 25 and 30

V/cm. However, the change in  $D_s$  ranged between 51.43 and 60.67 % while increasing the temperature from 80 to 95 °C and EFS from 15 to 30 V/cm. As the PPO contain Copper as prosthetic group therefore, EFS or the electric current generated during OH must have some additional effects on its inactivation than that of purely thermal treatment [44, 18]. The increase in EFS reduced the  $z$ -value indicating the non thermal effects of OH on PPO inactivation. Castro et al. [18]; Icier et al. [44, 45]; Brochier et al. [17]; Makroo et al. [65] and Saxena et al. [94] have also reported enhanced inactivation at higher EFS during OH in various food materials. The percent reduction in the  $z$  value was found to be 16.91 and 79.19 % in labile and resistant fraction respectively while increasing the EFS from 15 to 30 V/cm. As the distinct isozyme model suggests that the resistant fraction is resistant to heat but the higher percent reduction in the  $z$  value due to increase in EFS infer that resistant fraction is not resistant to the change in electric field as it is to the change in temperature.



**Fig. 4.22** Effect of EFS on rate constant of labile fraction of PPO ( $k_l$ ) at 80 ( $\square$ ), 85( $\circ$ ), 90 ( $\triangle$ ) and 95°C ( $\nabla$ )



**Fig. 4.23** Effect of EFS on rate constant of resistant fraction of PPO ( $k_r$ ) at 80 ( $\square$ ), 85( $\circ$ ), 90 ( $\triangle$ ) and 95°C ( $\nabla$ )

Table 4.15 The Distinct enzymes model coefficients and statistical parameters of PPO inactivation during OH

| EFS (Vcm <sup>1</sup> ) | Temperature (°C) | A <sub>1</sub> | k <sub>1</sub> | A <sub>r</sub> | k <sub>r</sub> | $\chi^2 (\times 10^{-3})$ | R <sup>2</sup> | F-value   | Prob>F |
|-------------------------|------------------|----------------|----------------|----------------|----------------|---------------------------|----------------|-----------|--------|
| 15                      | 80               | 0.782          | 0.513          | 0.217          | 0.034          | 1.080                     | 0.986          | 414.557   | 0.036  |
|                         | 85               | 0.768          | 0.569          | 0.231          | 0.158          | 0.004                     | 0.999          | 87964.753 | 0.002  |
|                         | 90               | 0.284          | 0.729          | 0.715          | 0.070          | 0.864                     | 0.991          | 433.884   | 0.035  |
|                         | 95               | 0.175          | 2.008          | 0.825          | 0.028          | 0.528                     | 0.995          | 625.457   | 0.029  |
| 20                      | 80               | 0.366          | 0.580          | 0.633          | 0.432          | 1.830                     | 0.978          | 220.316   | 0.049  |
|                         | 85               | 0.370          | 0.679          | 0.629          | 0.508          | 1.410                     | 0.985          | 268.556   | 0.044  |
|                         | 90               | 0.589          | 1.037          | 0.410          | 0.303          | 1.480                     | 0.985          | 241.726   | 0.047  |
|                         | 95               | 0.478          | 2.478          | 0.521          | 0.594          | 0.076                     | 0.999          | 4298.472  | 0.011  |
| 25                      | 80               | 0.581          | 0.686          | 0.418          | 0.531          | 0.501                     | 0.994          | 739.794   | 0.027  |
|                         | 85               | 0.530          | 1.036          | 0.469          | 0.515          | 0.235                     | 0.997          | 1453.439  | 0.019  |
|                         | 90               | 0.546          | 2.247          | 0.453          | 0.653          | 2.560                     | 0.978          | 128.415   | 0.064  |
|                         | 95               | 0.477          | 3.388          | 0.522          | 0.752          | 1.820                     | 0.986          | 169.235   | 0.056  |
| 30                      | 80               | 0.555          | 0.768          | 0.444          | 0.614          | 1.400                     | 0.986          | 251.739   | 0.046  |
|                         | 85               | 0.495          | 1.056          | 0.504          | 0.780          | 0.021                     | 0.999          | 15061.108 | 0.005  |
|                         | 90               | 0.512          | 2.602          | 0.487          | 0.876          | 1.290                     | 0.990          | 236.756   | 0.047  |
|                         | 95               | 0.475          | 3.969          | 0.524          | 0.900          | 2.180                     | 0.984          | 135.535   | 0.063  |



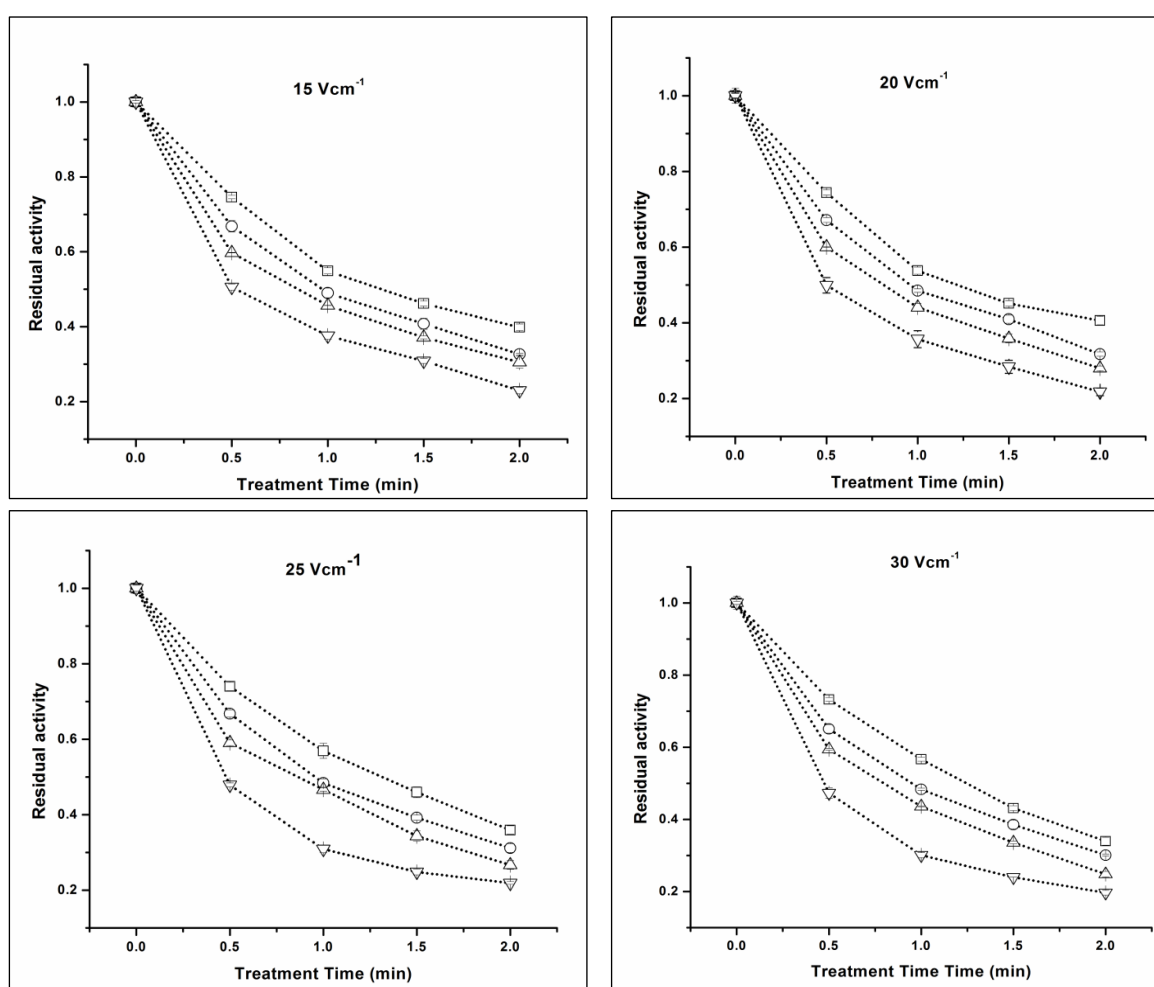
**Table 4.16** Decimal reduction time and Thermal resistance constant of labile (l) and resistant (r) fraction of PPO calculated from best fit model parameters

| EFS (Vcm <sup>1</sup> ) | Temperature (°C) | D <sub>l</sub> (min) | D <sub>r</sub> (min) | z <sub>l</sub> (°C) | z <sub>r</sub> (°C) |
|-------------------------|------------------|----------------------|----------------------|---------------------|---------------------|
| 15                      | 80               | 4.49                 | 67.74                | 25.31               | 177.89              |
|                         | 85               | 4.05                 | 14.58                |                     |                     |
|                         | 90               | 3.16                 | 32.90                |                     |                     |
|                         | 95               | 1.15                 | 82.25                |                     |                     |
| 20                      | 80               | 3.97                 | 5.33                 | 23.78               | 108.45              |
|                         | 85               | 3.39                 | 4.53                 |                     |                     |
|                         | 90               | 2.22                 | 7.60                 |                     |                     |
|                         | 95               | 0.93                 | 3.88                 |                     |                     |
| 25                      | 80               | 3.36                 | 4.34                 | 21.63               | 99.25               |
|                         | 85               | 2.22                 | 4.47                 |                     |                     |
|                         | 90               | 1.02                 | 3.53                 |                     |                     |
|                         | 95               | 0.68                 | 3.06                 |                     |                     |
| 30                      | 80               | 3.00                 | 3.75                 | 21.03               | 90.32               |
|                         | 85               | 2.18                 | 2.95                 |                     |                     |
|                         | 90               | 0.89                 | 2.63                 |                     |                     |
|                         | 95               | 0.58                 | 2.56                 |                     |                     |

#### 4.4.1.2 Peroxidase (POD) inactivation

Peroxidase (POD) is generally available in the form of isozymes and in presence of hydrogen peroxide it causes single electron oxidations of many compounds. The POD acts synergistically with PPO during the oxidation reaction of phenolic compounds catalyzed by PPO because of the production of hydrogen peroxide. Hence it is important to study the characteristics of POD in addition to the PPO of mango for their significance in the color preservation of the mango or its products [72]. The POD activity in the fresh mango puree was observed to be  $405.2 \pm 11.4$  units. Inactivation pattern of POD in terms of residual activity during OH under different temperature and EFS conditions are shown in Fig. 4.24. The chi-square and  $R^2$  values of the tested kinetic models Eq. (3.15) to (3.20) is listed in the Table 4.17 and the Weibull model was found to be the best fit for POD inactivation during. Goodness of fit for the best model can also be seen in Fig. 4.21(b) where the experimental and predicted values are between the 5 % confidence interval. Brochier et al. [17] also observed that the POD inactivation by OH in sugarcane juice was best described by Weibull kinetic model. Conventionally the degradation of microbes, enzymes and nutrients occurring during food processing and storage is described by 0<sup>th</sup>, 1<sup>st</sup> or higher order kinetics. However the Weibull distribution function has an interesting potential for these degradation, it has been found to describe non iso-thermal degradation

of enzymes, in addition to the rate constant this model include a constant for shape thus making this model highly flexible [73]. Peleg and Penchina [76] explained the Weibull distribution as it assumed that the sensitivity to the thermal treatment depends on the intensity of heat and enzyme activity achieved at that point of time, however it is independent of the rate of reduction of activity by which the residual activity has been achieved. The difference in the inactivation pattern among POD and PPO should be due the difference in their chemical structure. The model parameters are listed in Table 4.18, the value of “b” increases with EFS and treatment temp and the “n” decreased with the temperature during OH treatment, whereas the value of “b” increased with the EFS.



**Fig. 4.24 The POD inactivation (residual activity) during OH at different EFS and temperature ( $^{\circ}\text{C}$ ) 80 ( $\square$ ), 85( $\circ$ ), 90 ( $\triangle$ ) and 95( $\nabla$ )**

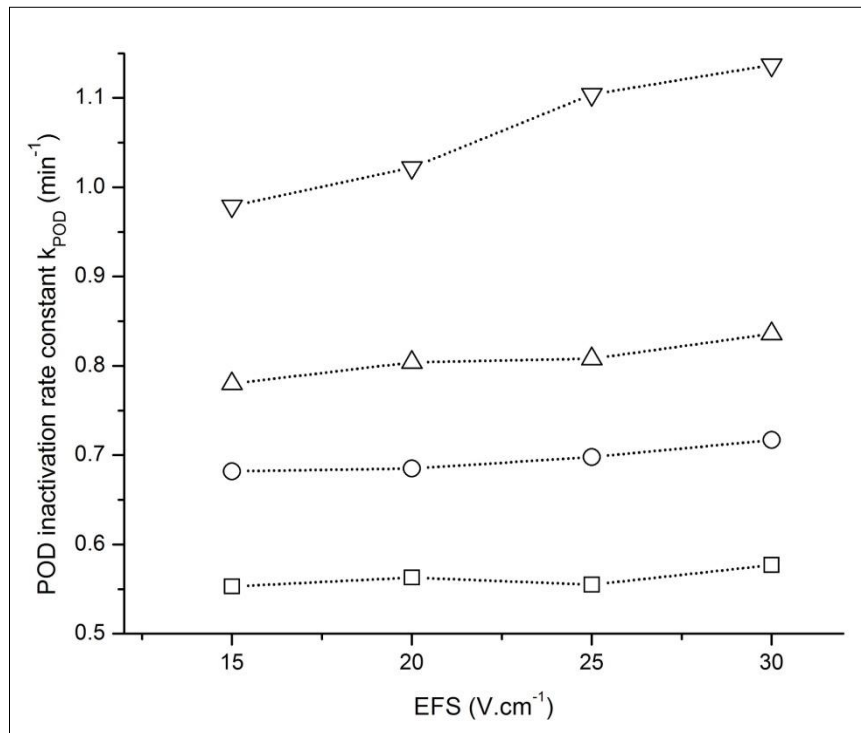
The D-value and z-value of the POD calculated from the rate content (Weibull model) is presented in Table 4.19. The reduction in D-value was recorded viz 4.15 and 13.89 % as the was changed EFS from 15 to 30 V/cm at 80 and 95 $^{\circ}\text{C}$  temperature respectively, the increase of 15 V/cm in EFS i.e. from 15 to 30 V/cm resulted in almost

10 °C reduction in z-value of POD during the OH. The effect of EFS on z-value reduction may be due to the higher extraction of POD from the tissue matrix of the mango puree followed by the inactivation by thermal effects.

**Table 4.17 Chi square and R<sup>2</sup> values of selected models for the POD inactivation**

| S No     | Model                       | $\chi^2$  | R <sup>2</sup>     |
|----------|-----------------------------|---|--------------------|
| 1        | First order                 | 2.112-9518.766  | 0.594-0.997        |
| 2        | Distinct isozymes           | 0.002-6.220   | 0.954-0.999        |
| 3        | Two-fraction                | 0.002-16.812  | 0.992-0.999        |
| 4        | Fractional conversion       | 0.337-51.694  | 0.957-0.999        |
| <b>5</b> | <b>Weibull distribution</b> | <b><math>0.2 \times 10^{-5}</math>-<math>43.2 \times 10^{-5}</math></b> | <b>0.992-0.999</b> |
| 6        | nth order                   | 0.190-4437.323  | 0.838-0.999        |

The effects of EFS on the rate of enzyme inactivation are shown in Fig. 4.25, very small changes in rate of inactivation was observed at lower temperature, however, increase in temperature caused higher effects of EFS on inactivation rate of the POD. The increase in EFS from 15 to 30 V/cm caused 4.33, 5.13, 7.17 and 16.13 % increase in inactivation rate of POD at 80, 85, 90 and 95 °C respectively. It can be assumed that the combined effect of temperature and EFS may be due to the advanced cell matrix damage at higher temperature and EFS causing relative higher cell rupture thus leads to higher enzyme release and consequently higher inactivation is achieved.



**Fig. 4.25 Effect of EFS on rate constant of POD at 80 (□), 85(○), 90 (△) and 95 °C (▽)**

**Table 4.18 Weibull model coefficients and statistical parameters of POD inactivation during OH**

| EFS (V/cm) | Temperature (°C) | b min <sup>-1</sup> | n     | $\chi^2 (\times 10^{-3})$ | R <sup>2</sup> | F value    | Prob>F                |
|------------|------------------|---------------------|-------|---------------------------|----------------|------------|-----------------------|
| 15         | 80°C             | 0.553               | 0.791 | 0.432                     | 0.992          | 2578.656   | 1.68×10 <sup>-5</sup> |
|            | 85°C             | 0.682               | 0.718 | 0.112                     | 0.998          | 8722.633   | 2.71×10 <sup>-6</sup> |
|            | 90°C             | 0.780               | 0.599 | 0.002                     | 0.999          | 375368.539 | 9.59×10 <sup>-9</sup> |
|            | 95°C             | 0.979               | 0.537 | 0.103                     | 0.998          | 7494.502   | 3.40×10 <sup>-6</sup> |
| 20         | 80°C             | 0.563               | 0.769 | 0.774                     | 0.987          | 1426.013   | 4.08×10 <sup>-5</sup> |
|            | 85°C             | 0.685               | 0.738 | 0.197                     | 0.997          | 4964.991   | 6.30×10 <sup>-6</sup> |
|            | 90°C             | 0.804               | 0.648 | 0.036                     | 0.999          | 24174.358  | 5.87×10 <sup>-7</sup> |
|            | 95°C             | 1.022               | 0.557 | 0.022                     | 0.999          | 34051.084  | 3.51×10 <sup>-7</sup> |
| 25         | 80°C             | 0.555               | 0.872 | 0.022                     | 0.999          | 49554.411  | 2.00×10 <sup>-7</sup> |
|            | 85°C             | 0.698               | 0.751 | 0.088                     | 0.998          | 10894.055  | 1.94×10 <sup>-6</sup> |
|            | 90°C             | 0.808               | 0.673 | 0.222                     | 0.997          | 3956.0638  | 8.85×10 <sup>-6</sup> |
|            | 95°C             | 1.104               | 0.534 | 0.321                     | 0.996          | 2232.5796  | 2.09×10 <sup>-5</sup> |
| 30         | 80°C             | 0.577               | 0.905 | 0.015                     | 0.999          | 69512.159  | 1.20×10 <sup>-7</sup> |
|            | 85°C             | 0.717               | 0.733 | 0.017                     | 0.999          | 53842.183  | 1.77×10 <sup>-7</sup> |
|            | 90°C             | 0.836               | 0.702 | 0.046                     | 0.999          | 18443.775  | 8.80×10 <sup>-7</sup> |
|            | 95°C             | 1.137               | 0.564 | 0.198                     | 0.998          | 3554.76515 | 1.04×10 <sup>-5</sup> |

**Table 4.19 Decimal reduction time (D value) and Thermal resistance constant (Z) of POD calculated from best fit model parameters**

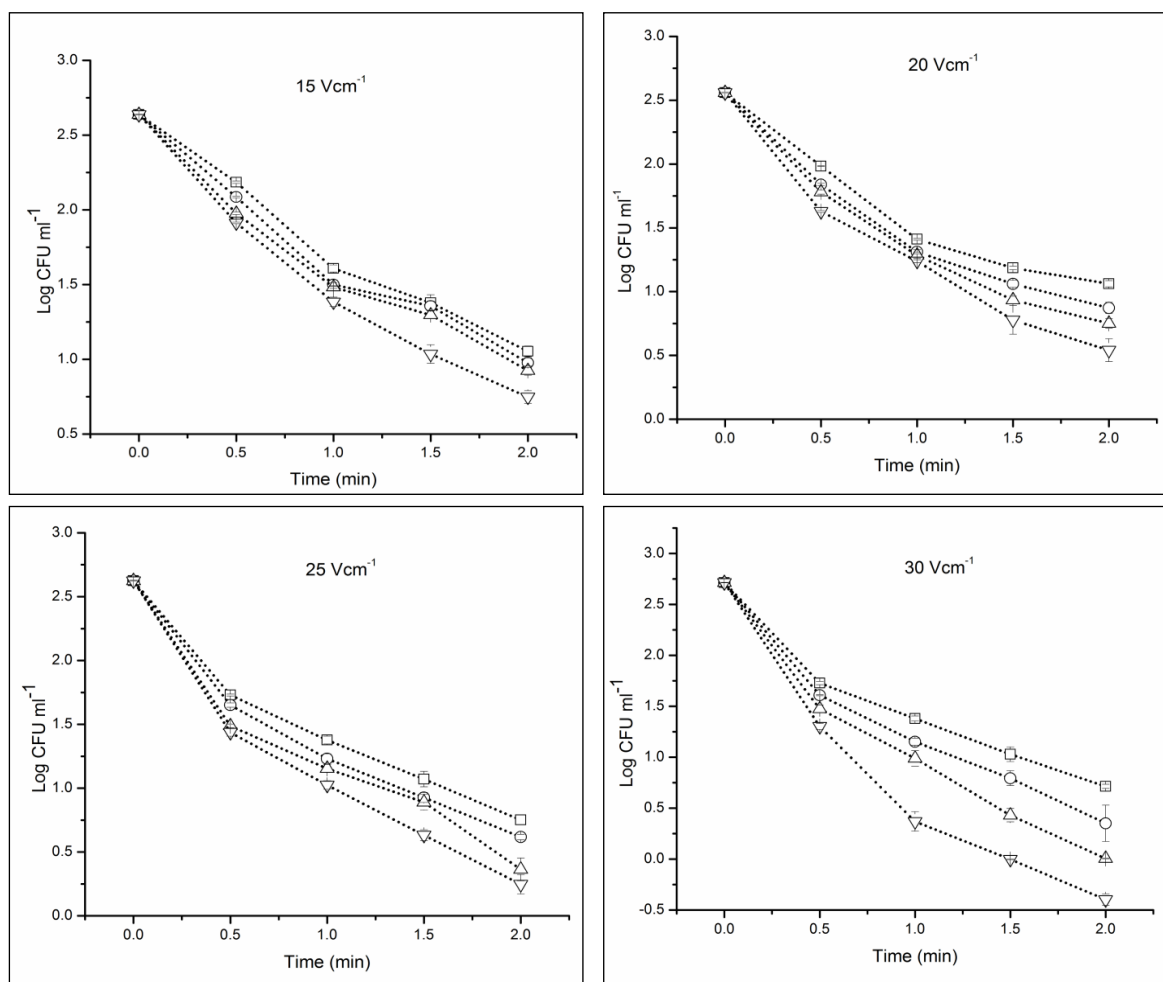
| EFS (V/cm) | Temperature (°C) | k <sub>POD</sub> | D <sub>POD</sub> (min) | Z <sub>POD</sub> (°C) |
|------------|------------------|------------------|------------------------|-----------------------|
| 15         | 80               | 0.553            | 4.16                   | 60.47                 |
|            | 85               | 0.682            | 3.38                   |                       |
|            | 90               | 0.780            | 2.95                   |                       |
|            | 95               | 0.979            | 2.35                   |                       |
| 20         | 80               | 0.563            | 4.09                   | 57.93                 |
|            | 85               | 0.685            | 3.36                   |                       |
|            | 90               | 0.804            | 2.86                   |                       |
|            | 95               | 1.022            | 2.25                   |                       |
| 25         | 80               | 0.555            | 4.15                   | 50.22                 |
|            | 85               | 0.698            | 3.30                   |                       |
|            | 90               | 0.808            | 2.85                   |                       |
|            | 95               | 1.104            | 2.09                   |                       |
| 30         | 80               | 0.577            | 3.99                   | 50.92                 |
|            | 85               | 0.717            | 3.21                   |                       |
|            | 90               | 0.836            | 2.75                   |                       |
|            | 95               | 1.137            | 2.03                   |                       |

#### 4.4.2 Bacterial inactivation kinetics

Ohmic heating of the food is a novel heating method where we use electric current to generate heat; it has the advantage of heating material rapidly and uniformly. Due these advantages it has a great scope for providing microbiologically safe and high-quality foods, and has wide capacity for applications such as sterilization, evaporation, blanching, fermentation, pasteurization [52]. The opinion on microbial inactivation during OH treatment was not reported in the same line. Sun et al. [107] reported that the OH treatment has shown some additional or non-thermal effect on microbial inactivation in milk, whereas Palaniappan and Sastry [74] did not get any significant evidence of non-thermal effects of OH in suspension of yeast cells in phosphate buffer solution. The microbial safety is one of the vital steps in food processing and preservation. Hence the inactivation of microbial population was studied in the present study. The initial count of the bacteria was quiet inconsistent and it ranged from 2.64 to 2.71 log CFU/mL, possibly due to the variations in sample like contamination, source and may be handling etc. The reduction in microbial population during OH at different conditions is shown in Fig. 4.26. The microbial population was observed to decrees with the treatment time and temperature. However it was also witnessed that the increase in EFS resulted in higher destruction of microbial cells. These results are in agreement with Sun et al. [106] and Kim and Kang [52, 53, 54]; Lee et al. [55] and Park and Kang [75] who conducted study to verify non thermal effects of OH on various pathogenic micro organisms such as *S. thermophilus*, *E. coli*, *S. typhimurium*, *L. monocytogenes* in milk, skim milk and cream; orange juice, salsa and apple juice respectively. Their results suggested that OH has some additional effects ion the microbial destruction due to the electrical effects. Sensoy et al. [99] also revealed that the increasing EFS cause the reduction in survival fraction of *Salmonella dublin* in skim milk. Ohmic heating resulted in severe morphological cell damage when compared with the conventional heating; the results were also proven by Transmission Electron Microscopy (TEM) analysis in buffered peptone water and apple juice [76]. The kinetic study revealed that the reduction in microbial population was following exponential pattern. The data was best fitted with the exponential model Eq. (3.15) to (3.20), and the model parameters are listed in Table 4.20. The value of reduction rate constant  $k$  ( $\text{min}^{-1}$ ) was increasing with increasing the temperature. Additionally, the increase in EFS also resulted in increase of  $k$  ( $\text{min}^{-1}$ ). These observations also signify the role of EFS or non-thermal effects of OH process on the microbial inactivation, although

the inactivation was majorly because of temperature or thermal effects, however the electrical effects were also present.

The activation energy was calculated from the Arrhenius equation using the information from the plot of  $\ln(k)$  and inverse of absolute temperature ( $T^{-1}$ ) Fig. 4.27, the linear regression data of the plots is listed in Table 4.21. The activation ranged from  $10.51 \pm 0.10$  to  $30.61 \pm 0.01$  kJ/mol, Hunt and Marinas [38] reported that the activation energy required for the inactivation of *E. coli* in an aqueous solution by ozone was 37.1 kJ/mol. The activation energy for TBC inactivation was observed to increase with the EFS during OH process (Fig. 4.28). However, the results recommended no significant difference in activation energy at 20 and 25 V/cm.



**Fig. 4.26** Semi-log plots for microbial reduction during OH at different EFS and temperature (°C) 80 (□), 85 (○), 90 (△) and 95 (▽)

Table 4.20 Exponential model parameters of microbial reduction during OH process

| EFS (V/cm) | Temperature (°C) | a     | -k min <sup>-1</sup> | χ <sup>2</sup> (10 <sup>-4</sup> ) | R <sup>2</sup> |
|------------|------------------|-------|----------------------|------------------------------------|----------------|
| 15         | 80               | 2.567 | 1.828                | 2.631                              | 0.976          |
|            | 85               | 2.521 | 1.864                | 2.954                              | 0.961          |
|            | 90               | 2.485 | 1.890                | 4.559                              | 0.958          |
|            | 95               | 2.475 | 2.142                | 2.002                              | 0.964          |
| 20         | 80               | 2.398 | 1.746                | 3.092                              | 0.924          |
|            | 85               | 2.358 | 1.911                | 3.226                              | 0.929          |
|            | 90               | 2.354 | 2.054                | 2.838                              | 0.939          |
|            | 95               | 2.325 | 2.243                | 3.513                              | 0.942          |
| 25         | 80               | 2.391 | 2.027                | 5.852                              | 0.934          |
|            | 85               | 2.358 | 2.181                | 3.139                              | 0.925          |
|            | 90               | 2.327 | 2.351                | 2.885                              | 0.917          |
|            | 95               | 2.304 | 2.556                | 1.364                              | 0.923          |
| 30         | 80               | 2.452 | 2.162                | 4.245                              | 0.928          |
|            | 85               | 2.428 | 2.533                | 1.494                              | 0.937          |
|            | 90               | 2.396 | 2.887                | 0.682                              | 0.938          |
|            | 95               | 2.271 | 3.318                | 0.035                              | 0.894          |

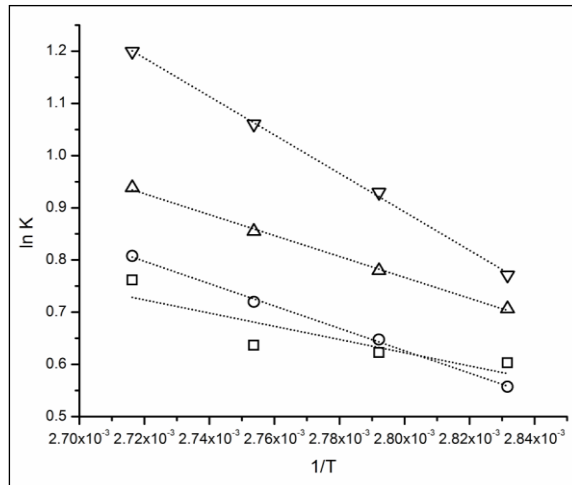


Fig. 4.27 Plot between inverse of absolute temperature (K) and reduction constant of microbial reduction during OH at 15 (□), 20 (○), 25 (△) and 30(▽) V/cm EFS

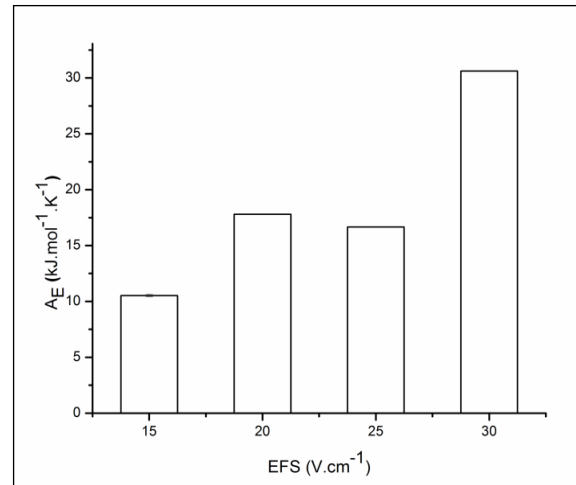


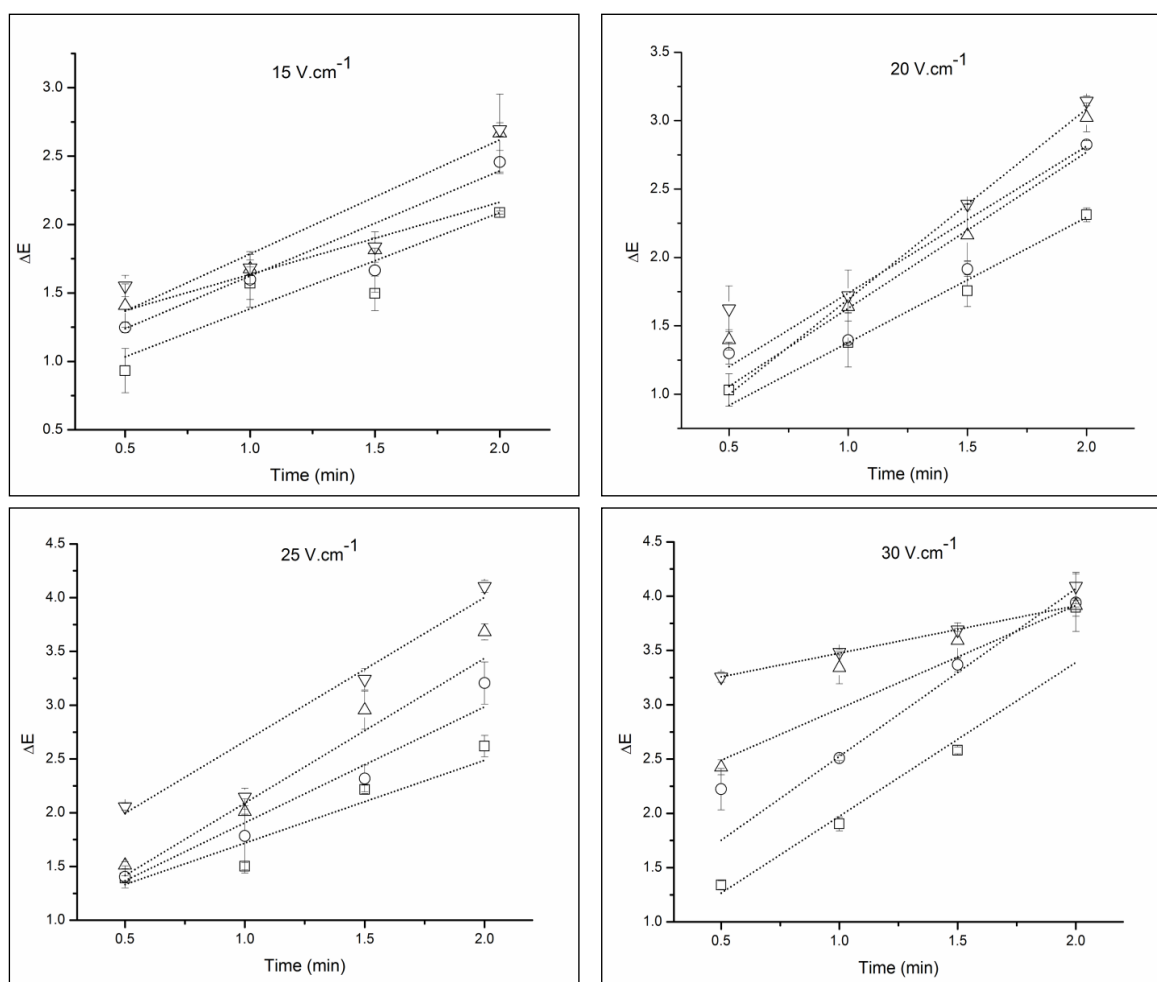
Fig. 4.28 Activation energy for microbial reduction at the different EFS of OH treatment

Table 4.21 Linear model parameters of T<sup>-1</sup> vs ln(k)

| EFS (V/cm) | Intercept | Slope    | R <sup>2</sup> |
|------------|-----------|----------|----------------|
| 15         | 4.164     | -1264.84 | 0.648          |
| 20         | 6.622     | -2141.54 | 0.997          |
| 25         | 6.375     | -2003.07 | 0.997          |
| 30         | 11.20     | -3682.39 | 0.998          |

#### 4.4.3 Color change ( $\Delta E$ )

Color is one of the important quality parameters of most of the fresh food items, for mango and its products color is being considered one of the prime attribute in addition to the quality parameters such as taste, aroma, flavor etc. Nevertheless, the change in color of the mango puree during processing is expected and may occur due to many reasons such as Maillard reaction, cooking, caramelization etc. The most critical reason for color change in mango and its products is the alterations in the pigment,  $\beta$ -carotenes during processing [2]. To evaluate the effect of various OH treatment on the color of mango puree, the color parameters,  $L^*$ ,  $a^*$  and  $b^*$  were measured by Hunter color Lab instrument followed by calculating the change in color ( $\Delta E$ ) using Eq. 3.14. The results of  $\Delta E$  during OH process at different temperature and EFS is shown in the Fig. 4.29.



**Fig. 4.29** Color change ( $\Delta E$ ) during OH at different EFS and temperature ( $^{\circ}\text{C}$ ) 80 ( $\square$ ), 85( $\circ$ ), 90 ( $\triangle$ ) and 95( $\nabla$ )

The  $\Delta E$  was increasing with the temperature at all the EFS, the change was observed to follow linear pattern with the heating time. As mentioned in the Table 4.22 the slope of



the linear regression was increasing with the temperature. At 30 V/cm, no change was observed in the change of  $\Delta E$  with the temperature. However, the  $\Delta E$  was increasing with increase in EFS. It indicates that the role of temperature in color degradation was lower at lower EFS whereas it has greater role at the higher EFS. Leizeron and Shimoni [57] observed the color change in orange juice in terms of browning index, the change in color would have been due to the non enzymatic browning reactions. Similar results were reported by Yildiz et al. [113] in pomegranate aril and whole fruit juice. They observed that, the value of hue angle was found to change with the heating time, however, the change was less during OH than conventional heating.

Mercali et al. [68] also observed the change in color of acerola pulp during OH, the possible reasons were reported to be the occurrence of electrochemical reactions. The electrolysis of water generates oxygen in the material and it catalyzes the degradation of coloring pigments or compounds of the material. It was also suggested that the higher change in color occurred at low frequency of electric current as compared to that at higher frequency (>100 Hz). The present study was conducted at a single frequency of 50 Hz therefore; no claim on such findings may be made. For better understanding of the color changes further research is required to be conducted.

**Table 4.22 Linear regression parameters of  $\Delta E$  during OH of mango puree**

| EFS (V/cm) | Temperature | Intercept | Slope | R <sup>2</sup> |
|------------|-------------|-----------|-------|----------------|
| 15         | 80          | 0.269     | 0.947 | 0.853          |
|            | 85          | 0.327     | 1.066 | 0.847          |
|            | 90          | 0.364     | 1.148 | 0.839          |
|            | 95          | 0.418     | 1.134 | 0.792          |
| 20         | 80          | 0.225     | 1.069 | 0.938          |
|            | 85          | 0.233     | 1.253 | 0.904          |
|            | 90          | 0.282     | 1.362 | 0.920          |
|            | 95          | 0.365     | 1.410 | 0.888          |
| 25         | 80          | 0.332     | 1.212 | 0.887          |
|            | 85          | 0.277     | 1.465 | 0.938          |
|            | 90          | 0.270     | 1.761 | 0.963          |
|            | 95          | 0.430     | 1.878 | 0.903          |
| 30         | 80          | 0.136     | 1.807 | 0.967          |
|            | 85          | 0.602     | 1.805 | 0.858          |
|            | 90          | 0.855     | 1.798 | 0.741          |
|            | 95          | 1.180     | 1.723 | 0.573          |

#### 4.5 Effects of heating and holding time of OH and hot water (HW) treatment

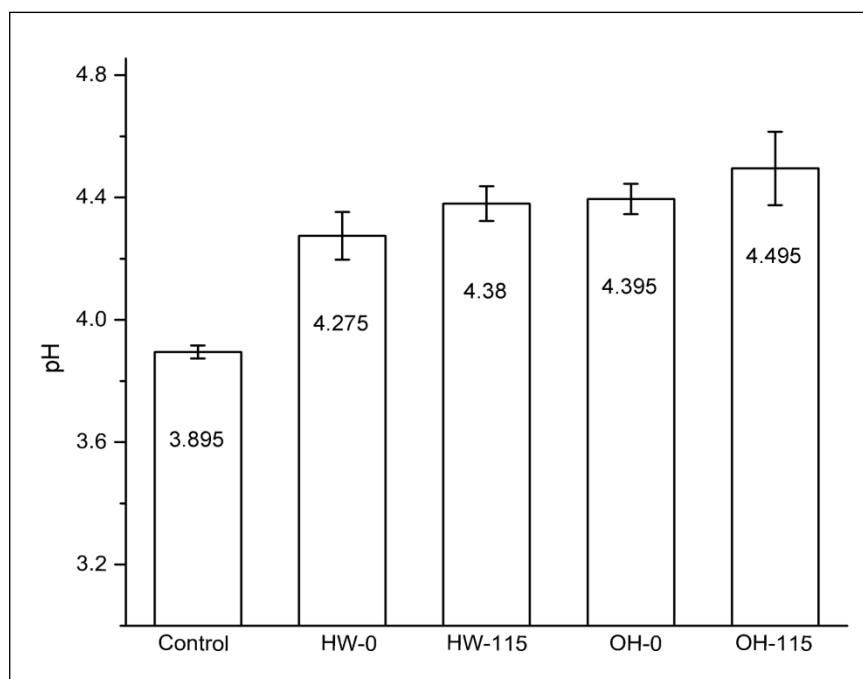
The effects of the OH and HW treatment were compared with the untreated samples (control) to verify the effects of heating of Come up Time (CUT) and the holding time of 115 s at 95 °C, the sample coding was as mentioned in Table 4.23

**Table 4.23 The sample coding for OH and HW treatment studies**

| Treatment | Treatment conditions   |
|-----------|--|
| Control   | With no treatment  |
| OH-0      | Heating up to 95 °C by OH method at 15 V/cm                                |
| HW-0      | Heating up to 95 °C by HW method   |
| OH-115    | Heating up to 95 °C and holding for 115 s at 95 °C by OH method at 15 V/cm |
| HW-115    | Heating up to 95 °C and holding for 115 s at 95 °C by HW method            |

##### 4.5.1 Titratable acidity, pH and total soluble solids (TSS)

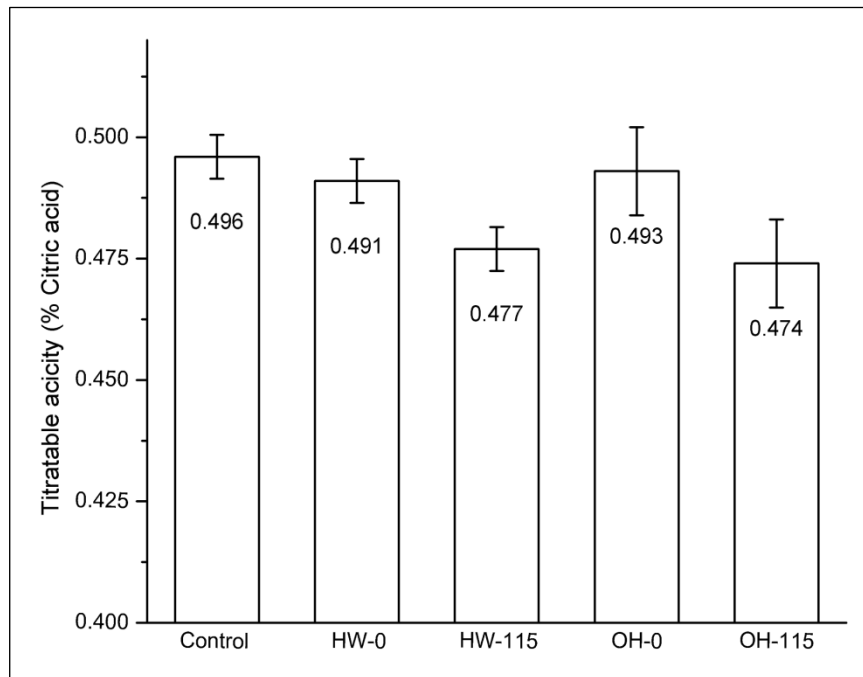
The pH and the titratable acidity of the fresh mango puree were found to be  $3.89 \pm 0.02$  and  $0.49 \pm 0.004$  % (citric acid), respectively. Similar results were also reported by Akhtar et al. [5]; Rajkumar et al. [80] and Kaushik et al. [50]. The change in pH and acidity during HW and OH treatment is shown in Fig. 4.30 and Fig. 4.31 respectively.



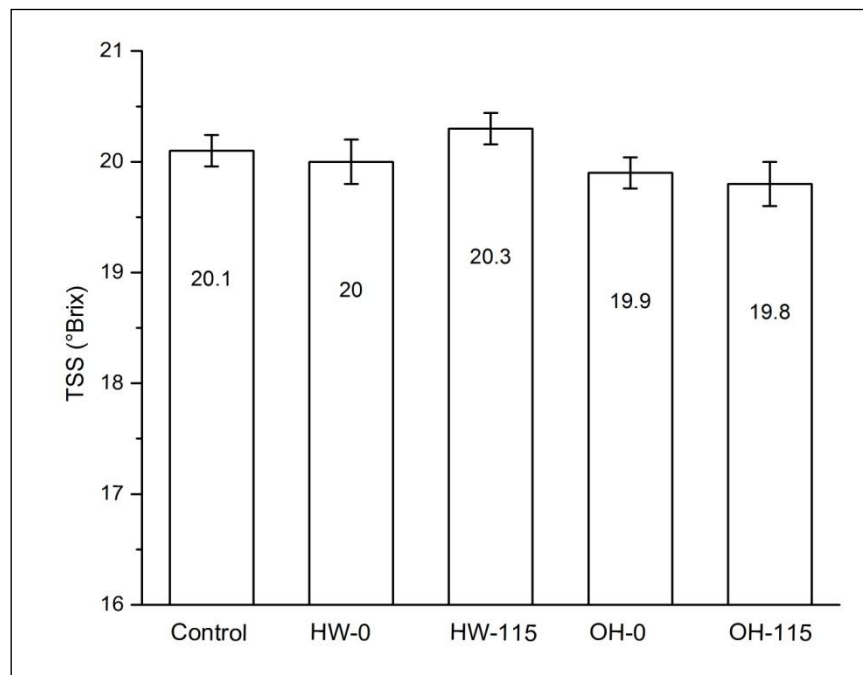
**Fig. 4.30 Effect of HW and OH treatment on pH of mango puree**

Vásquez-Caicedo et al. [110] also reported that the pH and acid content of mango puree and nectar changes during thermal treatment (at 80-93 °C). And Darvishi et al. [24] also reported the OH causes changes in pH of pomegranate juice. Because of very narrow difference between the pH of mango puree during CUT and holding period, it was difficult to

make any conclusion regarding the effect of CUT and holding period in the pH change of mango puree during HW and OH treatment in the present study. However, a higher change in pH and acidity were observed in OH treatment as compared to its HW counterpart. The higher changes in pH and acidity during ohmic heating may be due to some electro-chemical changes induced by the electric current during OH treatment.



**Fig. 4.31** Effect of HW and OH treatment on acidity (% citric acid) of mango puree

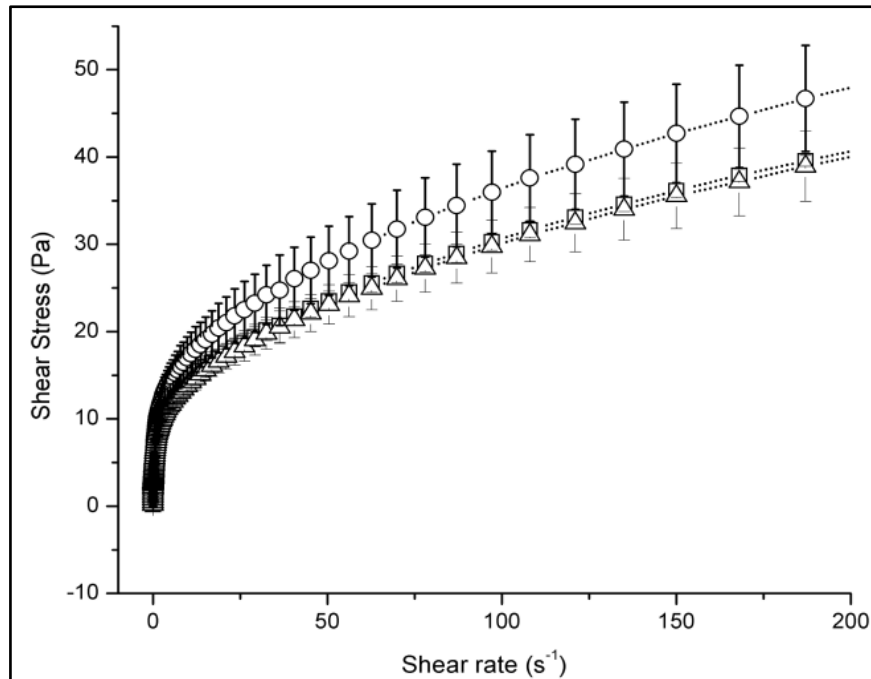


**Fig. 4.32** Effect of HW and OH treatment on TSS content of mango puree

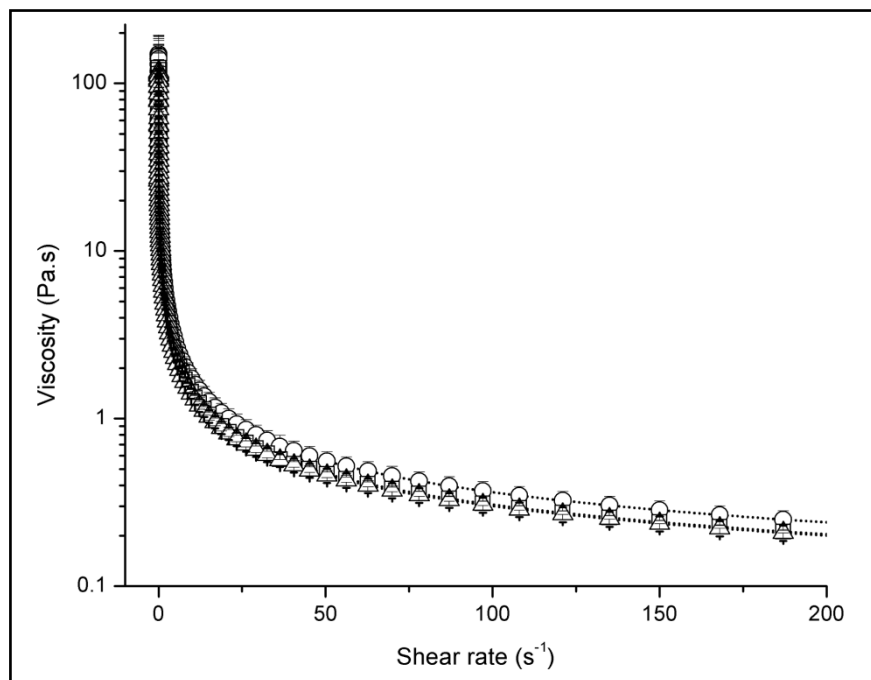
Additionally, the changes in pH or acidity may be due to the thermal degradation of heat sensitive organic acids present in mango such as ascorbic acid. The ascorbic acid, which is reported in literature a highly heat sensitive, on blanching or pasteurization it degrades to dehydroascorbic acid and degradation continuous further [81]. Additionally, conventional thermal treatment often causes some changes in foods, including loss of color, flavor, texture and functionality [73]. The total soluble solids did not show any major change during the CUT period or holding in either of the thermal treatment (HW or OH), the results are depicted in Fig. 4.32. The variation in results might be due to the compositional changes or possibly an error of experiment or slight moisture loss due to the evaporation of water at elevated temperature.

#### **4.5.2 Steady shear flow behavior of puree**

The flow behavior of the mango puree at 25 °C was observed to have a shear thinning or pseudoplastic behavior as shown in rheograms depicted as Fig. 4.33 and 4.34, similar results were observed by Ahmed et al. [3]; Guerrero and Alzamora [33] and Gundurao et al. [35]. Both the heat treatments OH and HW were found to cause slight changes in the flow pattern or slight reduction in the viscosity of the mango puree Fig. 4.34. The reduction in the viscosity was also reported by Ahmed et al. [3] during the high-pressure treatment of two varieties of mango puree. The solid fraction except the serum part of the mango puree is highly responsible for the viscosity and consistency of the mango puree, which majorly consists of cell wall components such as pectin and pectic substances. The damage caused by the thermal treatment to the solid fraction components of the mango puree may be the reason for the slight changes in the flow behavior of mango puree [40]. Lemmens et al. [58] reported that the thermal treatment causes cell separation in plant tissues, thus dissolving pectic substance which eventually results in the change of textural properties. The results were in good agreement with microscopy studies discussed in the following section.



**Fig. 4.33** Shear stress vs. shear rate plot of Control (○), HW (□) and OH (△) treated mango puree



**Fig. 4.34** Viscosity vs shear rate plot of Control (○), HW (□) and OH (△) treated mango puree

#### 4.5.2.1 Kinetics of steady shear flow behavior

The rheograms were analyzed for different models suggested Ahmad et al. [3], listed in Table 4.24. The best fit model was selected on the basis of least chi-square ( $\chi^2$ ) value and highest value of the coefficient of determination ( $R^2$ ), as the values are presented in Table 4.25 hence, the best model was found to be Herschel–Bulkley. This

model suggests that the mango puree need some yield stress to start the flow and later the flow will be shear thinning or pseudoplastic.

**Table 4.24 List of the models used to verify flow behavior of HW and OH treated mango puree [3]**

| S. No | Model            | Equation                             | Equation no |
|-------|------------------|--------------------------------------|-------------|
| 1.    | Newtonian        | $\sigma = k(\gamma)$                 | Eq.(4.10)   |
| 2.    | Power            | $\sigma = k(\gamma)^n$               | Eq.(4.11)   |
| 3.    | Casson model     | $\sigma = (k_0 + k(\gamma)^{0.5})^2$ | Eq.(4.12)   |
| 4.    | Bingham          | $\sigma = \sigma_0 + k(\gamma)$      | Eq.(4.13)   |
| 5.    | Herschel–Bulkley | $\sigma = \sigma_0 + k\gamma^n$      | Eq.(4.14)   |

Where:  
 $\sigma_0$  Yield stress (Pa)       $k$  Consistency coefficient (Pa.s<sup>n</sup>)  
 $\sigma$  Shear stress (Pa)       $\gamma$  Shear rate (s<sup>-1</sup>)       $k_0$  Constant consistency factor  
 n Flow behavior index

**Table 4.25 Model parameters and Co-efficient of determination (R<sup>2</sup>) and RMSE value of the fitted models**

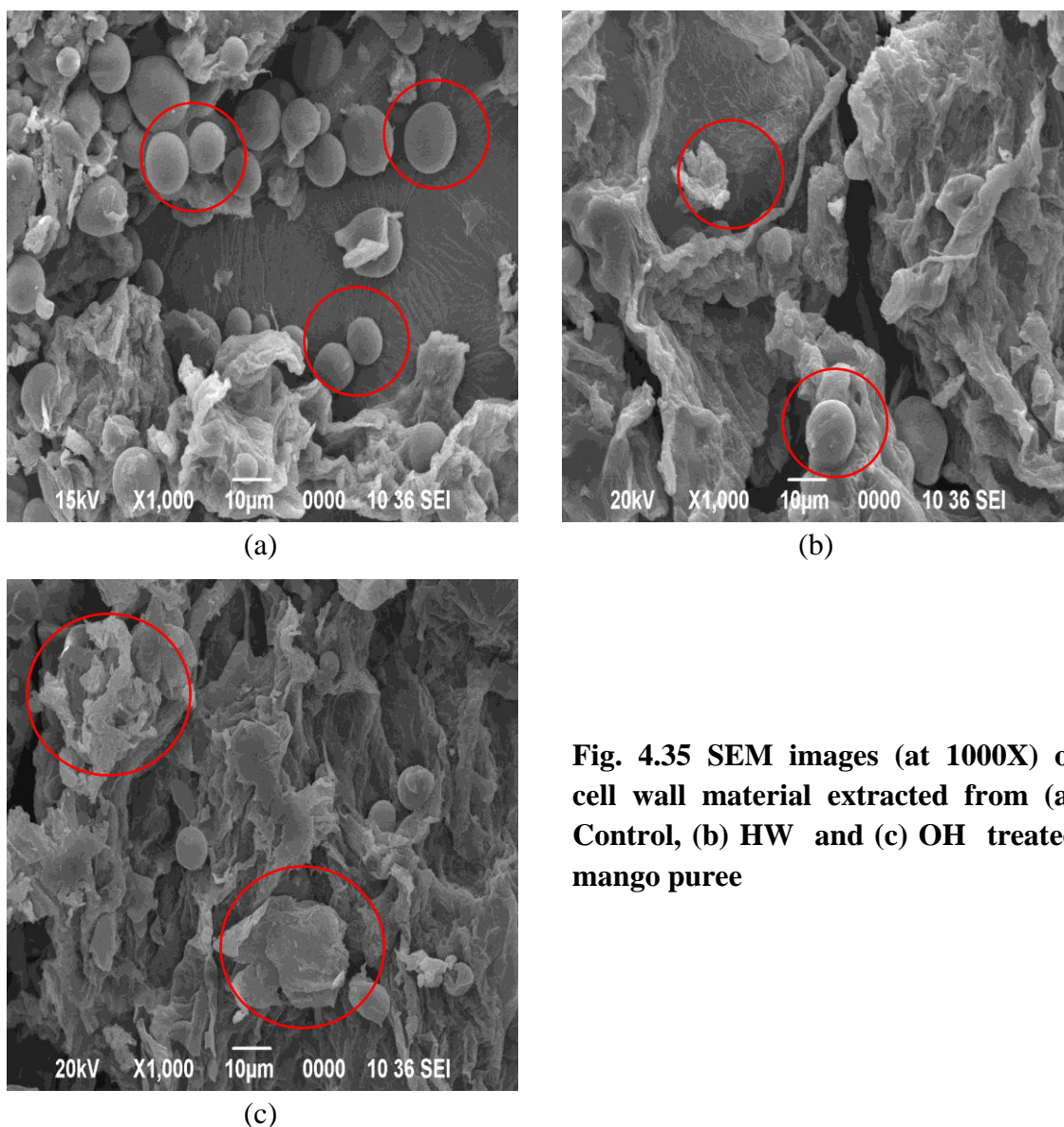
| Power model            |            |       |       |       |        |                |
|------------------------|------------|-------|-------|-------|--------|----------------|
| Sample                 | $\sigma_0$ | $k_0$ | $K$   | n     | RMSE   | R <sup>2</sup> |
| Con                    |            |       | 7.713 | 0.347 | 1.279  | 0.995          |
| HW-115                 |            |       | 6.620 | 0.345 | 1.368  | 0.991          |
| OH-115                 |            |       | 6.156 | 0.356 | 1.145  | 0.994          |
| Casson model           |            |       |       |       |        |                |
|                        |            |       | $K$   |       | RMSE   | R <sup>2</sup> |
| Con                    |            | 2.820 | 0.276 |       | 4.295  | 0.939          |
| HW-115                 |            | 2.620 | 0.253 |       | 3.398  | 0.946          |
| OH-115                 |            | 2.537 | 0.255 |       | 3.432  | 0.944          |
| Herschel–Bulkley model |            |       |       |       |        |                |
|                        | $\sigma_0$ |       | $k$   | n     | RMSE   | R <sup>2</sup> |
| Con                    | 1.837      |       | 6.144 | 0.383 | 1.064  | 0.996          |
| HW-115                 | 2.260      |       | 4.694 | 0.399 | 1.039  | 0.995          |
| OH-115                 | 1.732      |       | 4.713 | 0.398 | 0.908  | 0.996          |
| Bingham model          |            |       |       |       |        |                |
|                        | $\sigma_0$ |       | k     |       | RMSE   | R <sup>2</sup> |
| Con                    | 10.290     |       | 0.154 |       | 6.878  | 0.842          |
| HW-115                 | 8.886      |       | 0.131 |       | 5.587  | 0.854          |
| OH-115                 | 8.375      |       | 0.130 |       | 5.582  | 0.853          |
| Newtonian model        |            |       |       |       |        |                |
|                        |            |       | k     |       | RMSE   | R <sup>2</sup> |
| Con                    |            |       | 0.193 |       | 11.580 | 0.5483         |
| HW-115                 |            |       | 0.164 |       | 9.800  | 0.5449         |
| OH-115                 |            |       | 0.162 |       | 9.419  | 0.5757         |

The consistency coefficient ( $k$ ) of the fresh sample was found to be 6.144 Pa.s and the heat treatment caused in its slight reduction. The value calculated was 4.69 and 4.71 Pa.s for HW and OH respectively and no significant difference ( $p < 0.05$ ) was observed in

the 'k' between the type of treatment. Gundurao et al. [35] and Ahmed et al. [3] also reported similar observation regarding the flow behavior of mango pulp. However, the values of the model parameters are slightly different and the variations must be due to the compositional or varietal differences in the mango sample. Yield stress is one of the important quality parameters that characterize the properties of semi-solid foods. The yield stress was found to slightly increase in HW treatment whereas no significant change was observed by the OH treatment when compared with the fresh mango puree. Ahmed et al. [3] reported a linear relationship of yield stress and level of pressure in *Alphonso* mango pulp whereas no specific trend was reported in *Chousa* mango pulp.

#### 4.5.3 Morphology of mango puree particle phase

The effects of HW and OH treatment on the morphology of the particle fraction or cell wall material of mango puree samples were analyzed by the Scanning Electron Microscopy (SEM). The SEM images are shown in Fig. 4.35, the intactness of the cell wall components can be seen in the circled portion (red). The thermal treatments caused the rupture of cell wall components as can be seen in the images. However, the OH caused higher damages to the cell wall material of the mango puree when compared with the HW treatment. The enhanced rupture could be due to the electroporation due to the electric field applied during OH treatment. De Oliveira et al. [26] witnessed similar changes in the morphology of the pectic substances extracted from passion fruit peel waste by moderate electric field and conventional method of extraction. The pectin extracted by moderate electric field had a sharp and rough grooved appearance as compared to that obtained by conventional extraction relatively. The OH even at moderate temperature (<50 °C) was found to cause additional disintegration in the cell membrane of apple and potato tissues, therefore, result in the higher juice extraction rates as compared to the conventional heating method [77]. As discussed in the flow behavior above, the slight changes in the viscosity or consistency of the HW and OH treated mango puree might be due to such morphological changes in particle fraction. In a different, but a relevant study Lee et al. [56] observed that verified that the destruction of the *E. coli* vegetative cell in orange juice was higher in OH than in conventional heating due to the electroporation mechanism. Thus, recommended that OH resulted in the higher destruction of the *E. coli* cells when compared with conventional heating and termed these effects nonthermal effects of OH.



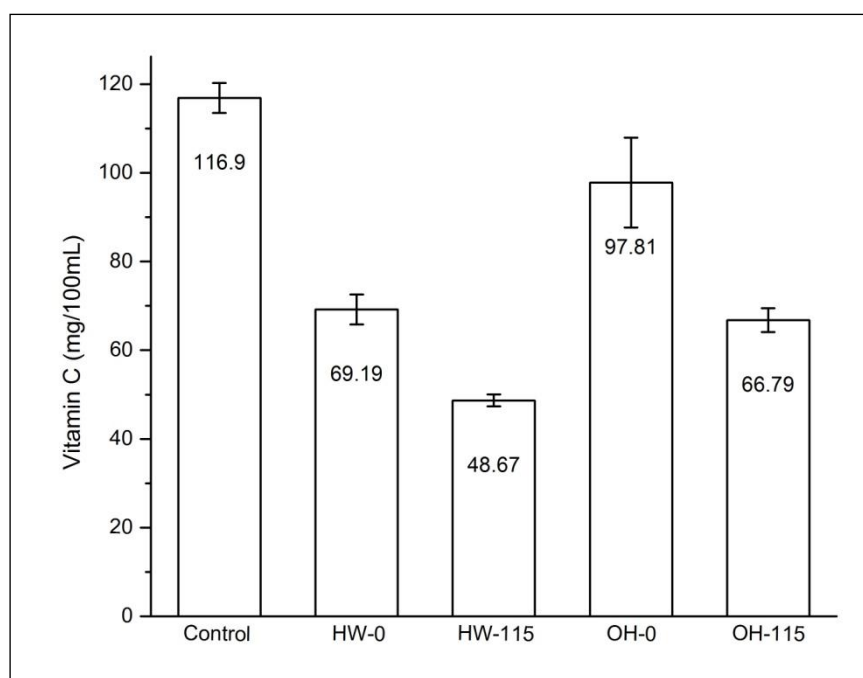
**Fig. 4.35 SEM images (at 1000X) of cell wall material extracted from (a) Control, (b) HW and (c) OH treated mango puree**

#### 4.5.4 Vitamin C

Ascorbic acid is a natural antioxidant, and it has a great role in human health by preventing serious diseases [83]. In addition to that, ascorbic acid also helps in the prevention of browning and shelf life enhancement of the product, provided it is present in enough amounts to consume all oxygen available in the product. The role of ascorbic acid is to regenerate quinones back to the parent phenols temporarily (caused due to PPO assisted oxidation) in a coupled oxido-reduction reaction, giving rise to dehydroascorbic acid and further degradation products [62]. However ascorbic acid is labile to elevated thermal treatments, therefore, the CUT period of HW-0 and OH-0 caused a loss of approximately 40 and 17 % in ascorbic acid respectively. The holding period of 115 s at 95 °C resulted in further loss up to 58 and 42 % in HW and OH treatments respectively as compared to control as indicated in Fig. 4.36. The lower damage of ascorbic acid during



OH should be due to the uniform heating and also during OH the pathway of ascorbic acid degradation gets interrupted. Louarme and Billaud [62] reported that OH did not generate 5-hydroxymethylfurfural (5-HMF) and had a low impact upon the formation of such sugars and ascorbic acid degradation products. Demirdöven and Baysal [27] witnessed the thermal destruction of ascorbic acid in orange juice due to both conventional as well as OH thermal treatments. In other findings Mercali et al. [68] observed the reduction of ascorbic acid following first order trend during conventional as well as OH in acerola pulp. However, increasing the frequency of OH treatment of acerola pulp can aid in reducing the degradation of ascorbic acid. Therefore, there is a scope to reduce the thermal damage to ascorbic acid by using OH at a higher frequency.

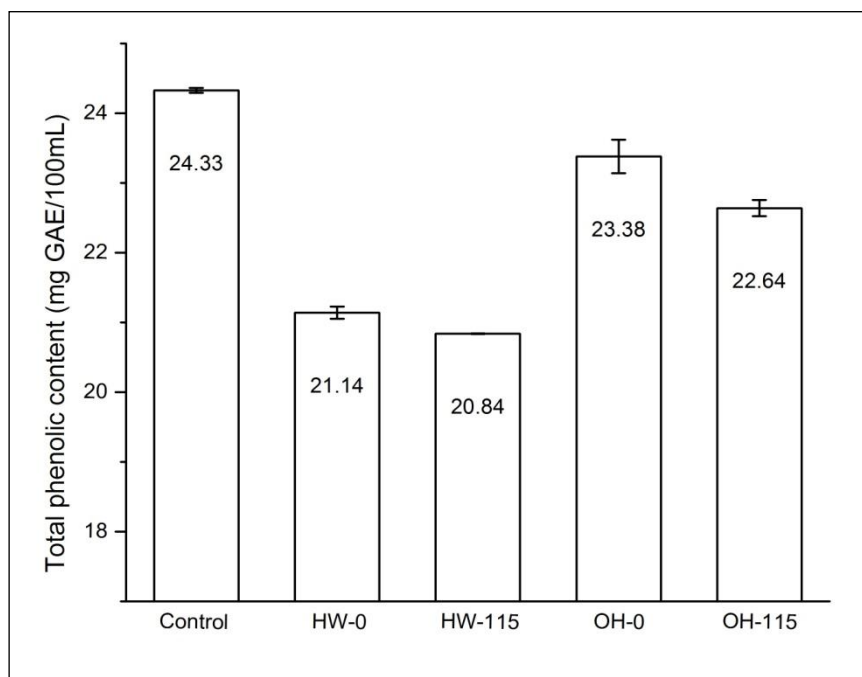


**Fig. 4.36 Effect of HW and OH treatment on Vitamin C content of mango puree**

#### 4.5.5 Total phenolic content (TPC)

The biological activity in cancer and heart diseases prevention of phenolic compounds present in fruits and vegetables have attracted great attention in recent times. Phenolic compounds are preferably oxidized in a biological medium and act as an antioxidant nutrient economizer, protecting organisms against the oxidative stress [83]. The presence of carotenes and phenolic compounds make mango products rich in antioxidant. However, the losses of these bioactive compounds due to processing need therefore to be minimized [97]. The present study showed (Fig. 4.37) higher TPC retention by the application of OH than HW treatment in mango puree, the observations

are in line with Liu et al. [60] suggesting that electric field caused greater disruption of cell membrane hence increased the TPC as compared to that of HW. However, the reduction in TPC due to either of the treatment as compared to the control should be due to the thermal damage to the heat-sensitive phenolic compounds [16]. The TPC loss during the CUT period of HW and OH treatment was found to be 13.07 % and less than 5 % respectively. However heating further for holding time caused additional reduction but the reduction was relatively lower than caused by CUT. The difference between TPC in HW-0 and HW-115 was observed to be insignificant ( $p < 0.05$ ), whereas significant differences were observed in OH-0 and OH-115. The TPC further reduced from 3.86 to 6.94 %, and the possible reason for the loss in holding time during OH might be due to the electrical effects of OH treatment or preferably the combination of the electric field with elevated temperature.

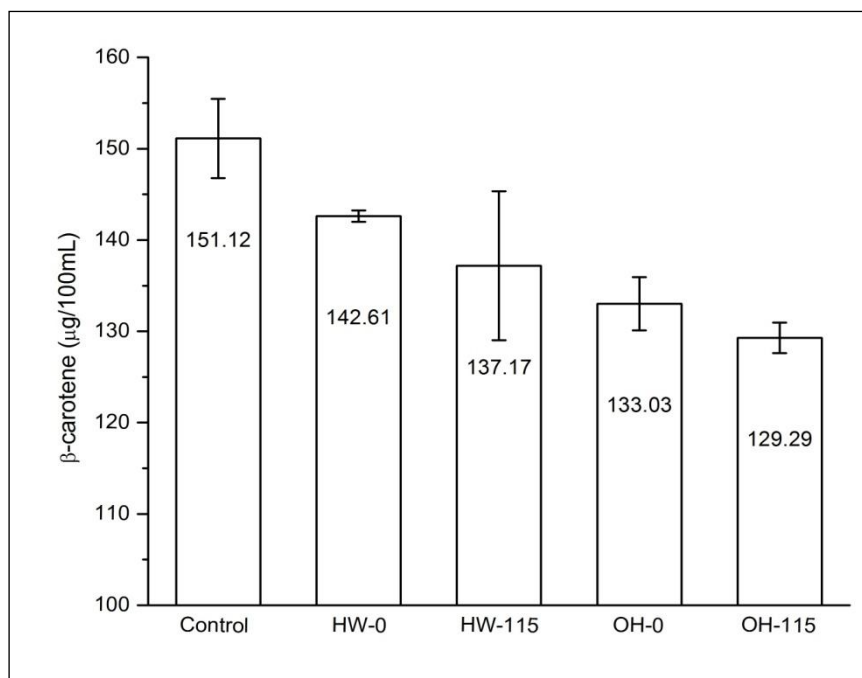


**Fig. 4.37 Effect of HW and OH treatment on the Total phenolic content of mango puree**

#### 4.5.6 $\beta$ -Carotenes

The bright yellowish orange color of the mango pulp is majorly due to the presence of  $\beta$ -carotenes [3].  $\beta$ -Carotenes are the major contributors to the nutritional quality of mangoes. The peculiar chemical structure of  $\beta$ -carotenes makes them essential micronutrient thus, extremely important for human health. For example, it has been shown that  $\beta$ -carotene possesses high pro-vitamin A activity and antioxidant capacity [58]. However, the processing conditions may change its structure to the cis-isomers,

which reduces its pro-vitamin A and antioxidant potential. The effect of CUT and holding period of HW and OH treatment on  $\beta$ -carotene of mango puree is shown in Fig. 4.38. The OH was observed to result in a higher reduction in  $\beta$ -carotene than reduction resulted by the HW treatment. The CUT resulted in reduction of 5.63 and 11.93 % loss in  $\beta$ -carotene of mango puree respectively. Whereas further holding at 95 °C for 115 s caused an additional loss in  $\beta$ -carotene, the loss was observed to be 9.23 and 14.44 % in HW-115 and OH-115 respectively when compared with the control. The thermal treatment of 4 min at 88-93 °C resulted in 3.14-9.75 % degradation of total  $\beta$ -carotenes in mango puree [111], and it was reported that the  $\beta$ -carotene degradation or conversion to the cis-isoform depends on both, the level of temperature as well as the heating time at the lethal temperature [58]. The present study witnessed higher degree in  $\beta$ -carotene degradation during OH as compared to that of HW heating method. Although, authors believe that the higher reduction during OH may be due to the electrical effects on the structure of  $\beta$ -carotene. However, to their best knowledge, no proper explanation is available in the literature for such an effect therefore, further investigation is recommended to identify the reason for such observations.



**Fig. 4.38** Effect of HW and OH treatment on  $\beta$ -carotenes of mango puree

#### 4.5.7 Storage study of HW and OH treated mango puree

The mango puree samples were treated by HW and OH at optimized conditions and filled hot in glass bottles followed by instant cooling. The glass vials filled with treated

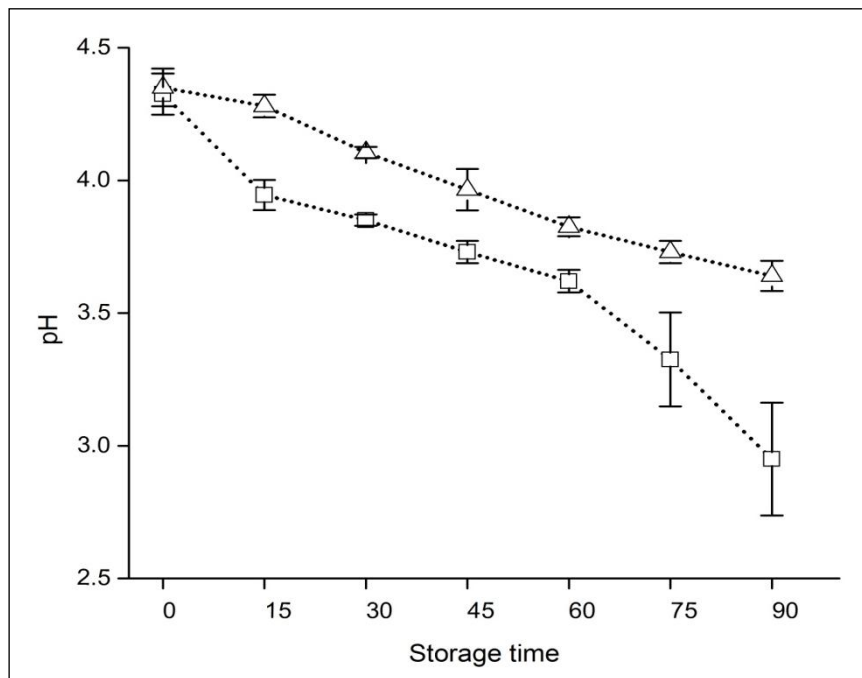
mango puree were stored for 3-months ambient conditions ( $25\pm 2$  °C) and away from direct sunlight. During the storage, the samples were analyzed for various parameters (discussed in following sections) at an interval of 15 days.

#### 4.5.7.1 Changes in pH, Titratable acidity and TSS

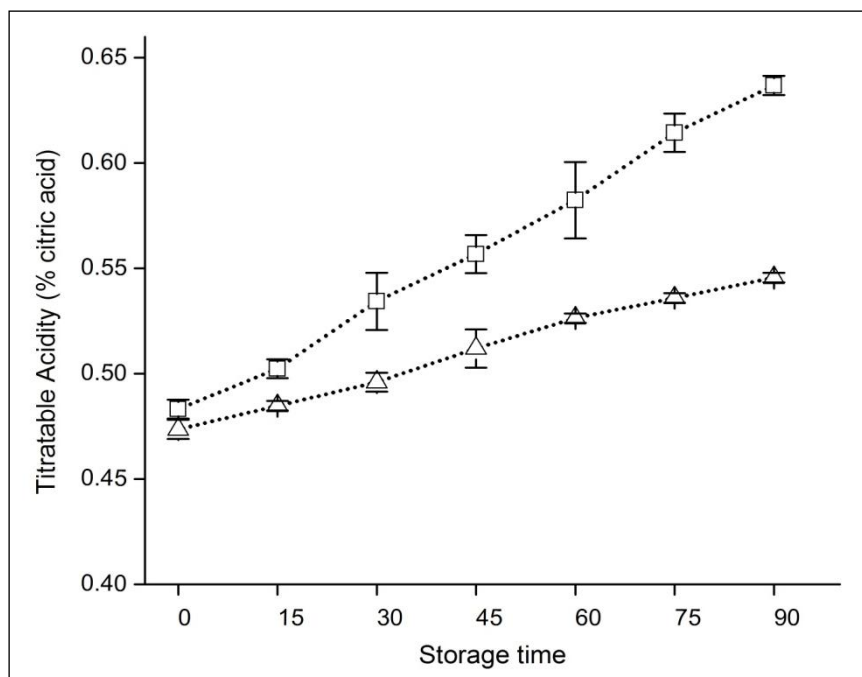
The pH in mango puree treated with HW and OH was found to reduce significantly ( $p < 0.05$ ) during the storage. The reduction in pH was found to be higher in HW treatment as compared to that of OH treatment; the differences are plotted and shown in Fig. 4.39. The reduction in pH was in good synchronization with the increase in acidity (Fig. 4.40) during the storage. During the storage period of 90 days, the pH reduced to 2.95 and 3.64, whereas the acidity increased to 0.636 and 0.545 % in HW and OH treated samples respectively. Guan et al. [32] also reported the reduction in pH and increase in the acidity of mango juice processed by high hydrostatic pressure and thermal treatment during the storage of 60 days. The change in the pH and acidity was relatively small due to the storage conditions, which they kept refrigerated whereas the present study was conducted at ambient conditions. Also, the differences may be due to the application of different technology and sample. During storage as the significant increase in bacterial count was witnessed (Fig. 4.49). Therefore, the increase in acidity of HW and OH treated samples may be due to the growth of acid-forming bacteria. Also, increase in acidity during storage could be attributed to the reaction of basic amines to convert into the compounds of lower basicity and to the degradation of sugars into acids during the Maillard reaction [60].

The major sugars present in mango pulp and puree are sucrose, glucose and fructose in addition to them it also contains small fractions of cellulose, hemicellulose and pectin [71]. And these are majorly responsible for the soluble solids in mango puree. The TSS of the mango puree didn't show any significant change in the storage time; the results are depicted in Fig. 4.41. Demirdöven and Baysal [27] also reported minor changes in soluble solids during six months storage of conventionally, Ohmically and microwave heat treated orange juice samples. However the soluble solids showed an abrupt decrease from 13 °Brix to near 6 °Brix in electropulsation treated orange juice after 60 days of storage, the decrease was reported due to the breakage of pectin substance by yeast and mold growing in the samples. The dielectric and HW heated whole mango fruits also showed no significant change in TSS during storage [105]. There was not any distinct difference in the TSS change in between the type of treatment (HW or OH). However,

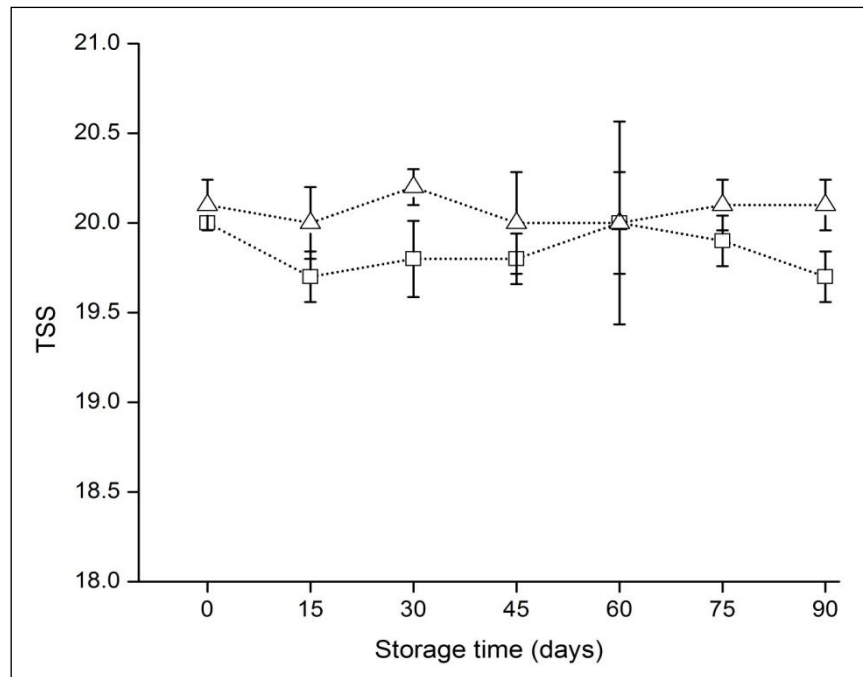
some observations had some deflection which could be due to experimental error or due to the fluctuation in the temperature at the time of measurement.



**Fig. 4.39** Change in pH of HW (□) and OH (△) treated mango puree sample during storage at ambient conditions



**Fig. 4.40** Change in acidity (% citric acid) of HW (□) and OH (△) treated mango puree sample during storage at ambient conditions

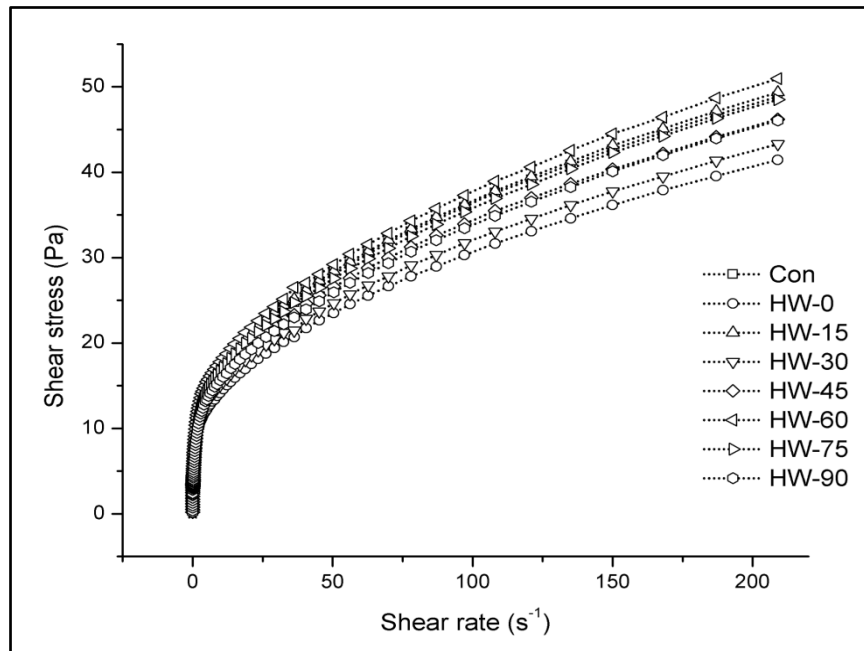


**Fig. 4.41** Change in TSS of HW ( $\square$ ) and OH ( $\Delta$ ) treated mango puree sample during storage at ambient conditions

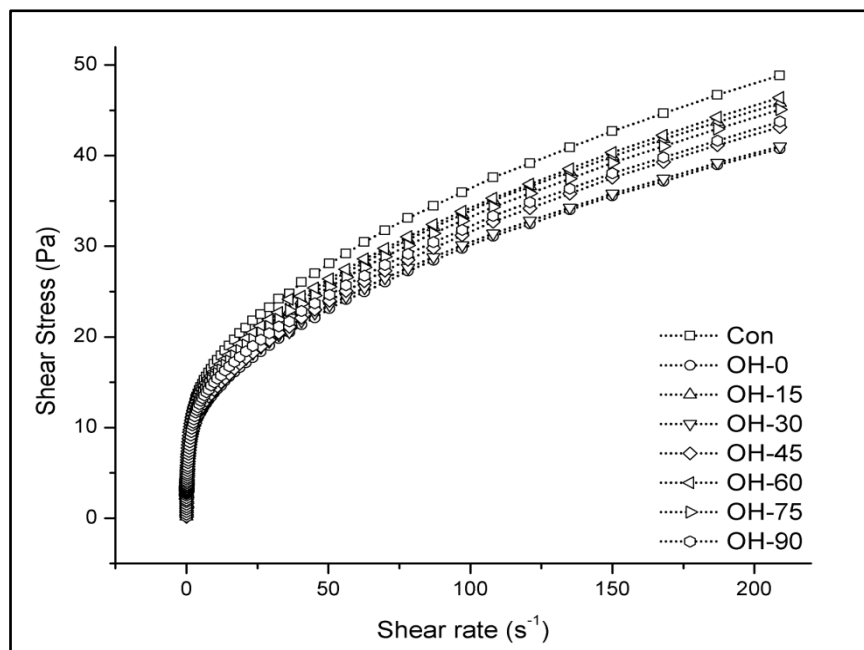
#### 4.5.7.2 Changes in steady shear flow behavior

Generally, fruit purees behave as non-Newtonian fluid due to the complex interactions among different components such as soluble sugars, pectic substances and suspended solids [9]. The rheograms of OH and HW treated mango puree are shown in Fig. 4.42-4.45. The steady shear flow behavior during storage was same as that of fresh puree thus the flow during the entire storage period was symmetrical to the Herschel-Bulkley model [33, 35], and the model parameters during storage are listed in Table 4.26. There was no significant difference observed in the flow behavior index ‘ $n$ ’ and consistency coefficient ‘ $k$ ’ in either of the treated mango puree during the storage. However, it was found that the yield stress ( $\sigma_0$ ) continuously decreased with the storage time in both the treated samples as shown in Table 4.26. In terms of industrial applications, the yield stress of food material is an important parameter and the maximum information regarding the yield stress could be helpful for the optimal design of the thermal processing unit of the food. Balestra et al. [9] has explained the change in the rheological behavior of different fruit purees during storage as a result of structural changes in the molecules due to the hydrodynamic forces generated and the increased alignment of the constituent molecules. The enzyme and microbial action on the dispersed phase (particle phase) including the cell walls, insoluble polymer clusters, and chains [28] should also have a good contribution in the yield stress reduction during storage. Although the TSS showed no major change during storage, the inter-linkage of

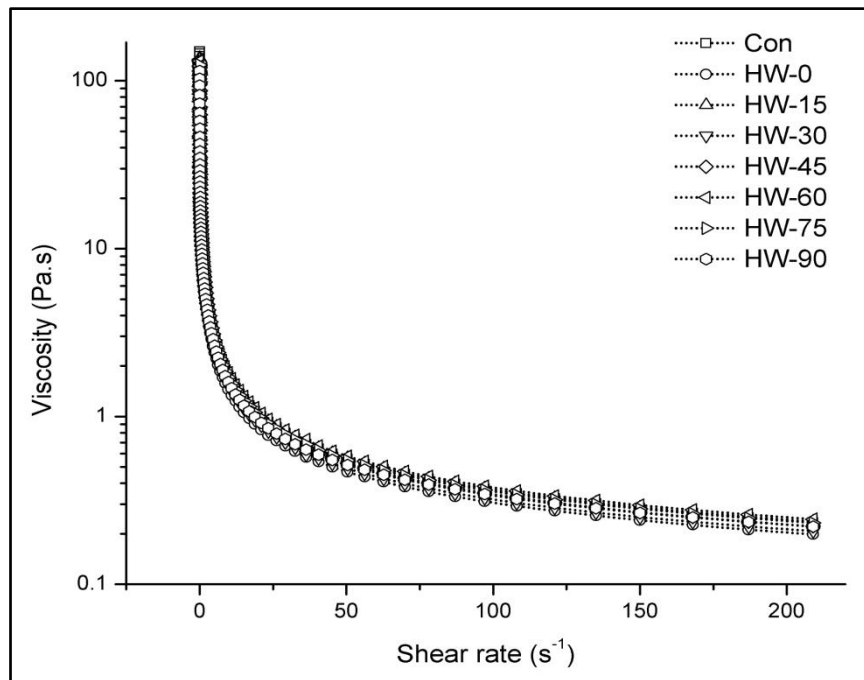
molecules might have got disturbed due to the change in acidity such as  $\beta$ -elimination degradation of pectic substances thus, results in the yield stress reduction. Thermally treated guava pulp was stored for 30 weeks by Harananan et al. [36] and significant reduction in yield stress (4-25 %) resulted during the storage at ambient conditions, in the present study the reduction in yield stress was calculated to be 8.3 – 31.37 % in HW and 8.90-41.01 % in OH treated samples.



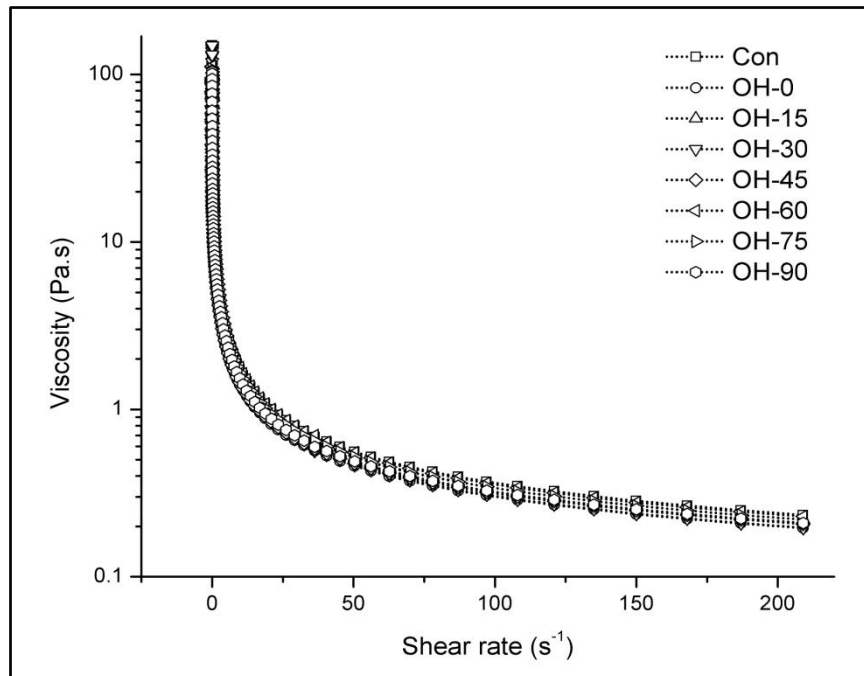
**Fig. 4.42 Shear rate vs. Shear stress plot of HW treated samples during storage, Con=control and (HW=Hot water, XX=storage days)**



**Fig. 4.43 Shear rate vs. Shear stress plot of OH treated samples during storage, Con=control and (OH=Ohmic Heated, XX=storage days)**



**Fig. 4.44** Shear rate vs. viscosity plot of HW treated samples during storage, Con=control and (HW=Hot water, XX=storage days)



**Fig. 4.45** Shear rate vs. viscosity plot of OH treated samples during storage, Con=control and (OH=Ohmic Heated, XX=storage days)



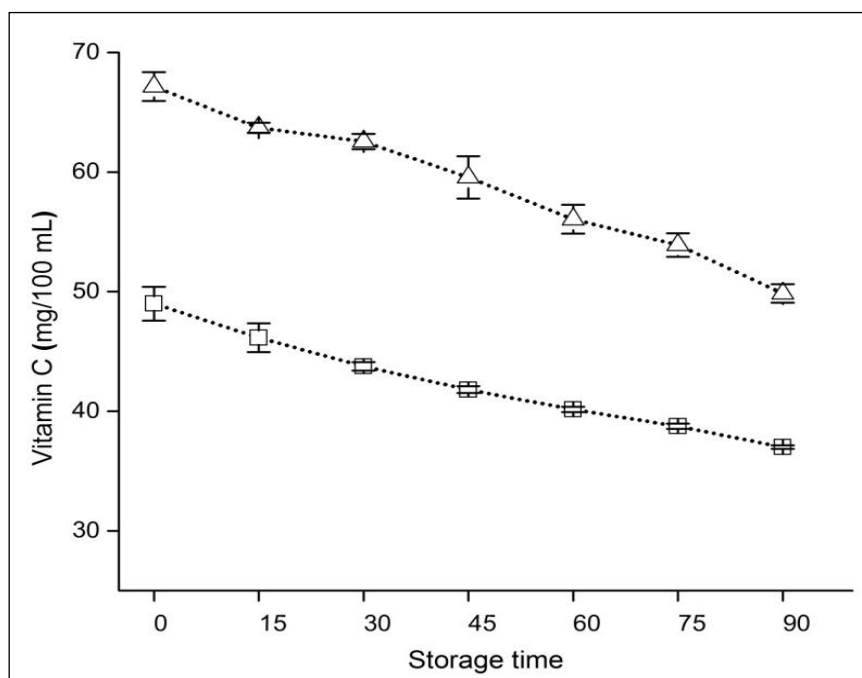
Table 4.26 Effect of storage time on Herschel–Bulkley model parameters

|            | Storage time (Days) | $\sigma_0$ (Pa) | $k$ (Pa.s <sup>n</sup> ) | $n$   | $R^2$ |
|------------|---------------------|-----------------|--------------------------|-------|-------|
| HW treated | 0                   | 2.260           | 4.694                    | 0.399 | 0.995 |
|            | 15                  | 2.072           | 6.099                    | 0.385 | 0.996 |
|            | 30                  | 2.010           | 5.677                    | 0.391 | 0.995 |
|            | 45                  | 1.735           | 5.702                    | 0.386 | 0.997 |
|            | 60                  | 1.687           | 5.536                    | 0.380 | 0.996 |
|            | 75                  | 1.612           | 5.426                    | 0.388 | 0.997 |
|            | 90                  | 1.551           | 5.453                    | 0.395 | 0.996 |
| OH treated | 0                   | 2.492           | 5.150                    | 0.404 | 0.996 |
|            | 15                  | 2.270           | 4.797                    | 0.393 | 0.995 |
|            | 30                  | 1.732           | 4.713                    | 0.398 | 0.996 |
|            | 45                  | 1.686           | 4.707                    | 0.409 | 0.996 |
|            | 60                  | 1.555           | 5.466                    | 0.393 | 0.997 |
|            | 75                  | 1.512           | 5.216                    | 0.395 | 0.997 |
|            | 90                  | 1.470           | 5.244                    | 0.393 | 0.996 |

#### 4.5.7.3 Changes in Vitamin C content during storage

Vitamins are among the most sensitive food components; degrade quickly due to many factors such as by oxygen-dependent reaction that continues until the oxygen is depleted [81]. Vitamin C is thermolabile and highly sensitive to various processing conditions, it gets readily oxidized and lost during storage, its degradation follows aerobic and/or anaerobic pathways and depends upon several processing conditions and the rate of reduction depends upon the storage conditions such as time and temperature [48,109]. Therefore, in the present study, the vitamin C content showed significant reduction during storage in both HW and OH treated samples. The reduction during storage in HW and OH is depicted in Fig. 4.48, the reduction among the treatment methods was not significant as the degradation was calculated to be 24.49 and 25.76 % of vitamin C content at the date of processing and packaging in HW and OH after three months respectively. As the OH treatment was found the result in lesser degradation of vitamin C as compared to control, consequently higher content was retained in OH as compared HW treated samples. Hymavathi and Khader [39] stored the vacuum dried mango powder in different packaging materials for six months, the reduction in ascorbic was extremely higher (17-81 %) as compared to our figures which must be due to the higher surface area of powder particles thus making them highly unstable. Kabasakalis et al. [48] also reported an ascorbic acid reduction in orange juice however the reduction under ambient storage condition was higher than that of the refrigerated condition. Choi et al. [21] has reported

degradation of ascorbic acid in fresh blood orange juice at the rate of 18 % per week stored under refrigerated conditions, whereas blood orange juice fortified juice showed only 10 % per week rate in ascorbic acid degradation. Tiwari et al. [109] did 30-days storage study of sonicated (2-10 min) and thermally pasteurized orange juice, after the 30 days of storage the loss in ascorbic acid was observed to be 19.76, 21.34 and 11.85 % in the orange juice sonicated at 0.33, 0.47 and 0.88 W/mL respectively, whereas the loss in thermally processed (98 °C for 21 s) was found to be 74.08 %.

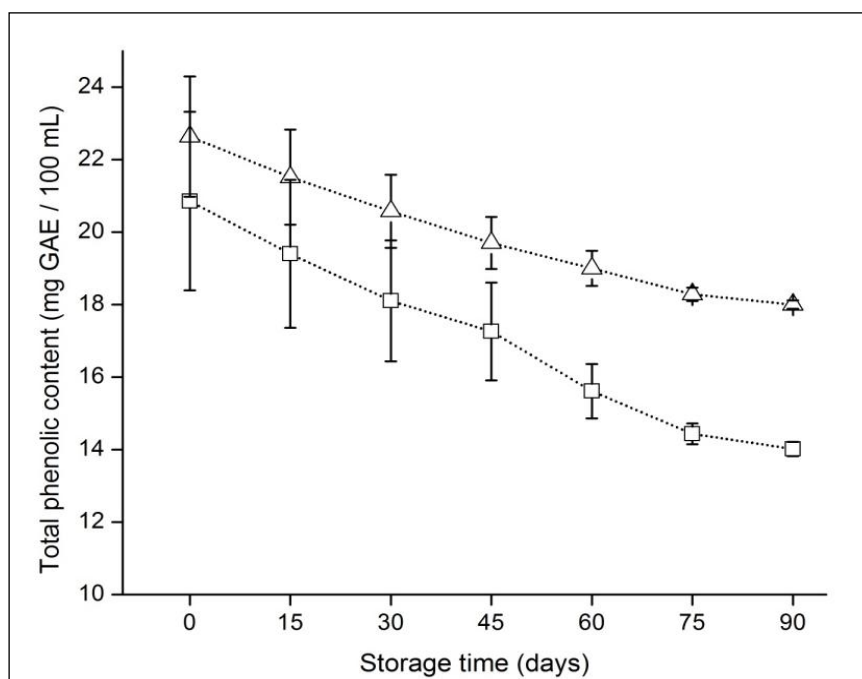


**Fig. 4.46 Change in Vitamin C content of HW (□) and OH (△) treated mango puree sample during storage at ambient conditions**

#### 4.5.7.4 Changes in Total phenolic content (TPC)

The antioxidant property of phenolic compounds helps in protecting various important qualities of food such as flavor and color degradation while protecting vitamin destruction during the storage. Also, these compounds bear health benefits to humans due to free radical scavenging activity that helps in the prevention and cure of various serious diseases [88]. The present study showed the significant decrease in the TPC of mango puree during the storage as shown in Fig. 4.46. As the OH was found to retain higher amount of TPC after the treatment, thus OH treated samples retained relatively higher amount of TPC than that of HW treated samples. TPC decreased progressively in mango nectar during storage at 4 and 25 °C, High-pressure treatment was reported to result in higher retention of TPC than high-temperature short temperature conventional thermal treatment [60]. Siddiq et al. [102] also reported a slight but significant reduction in TPC

of fresh-cut mango treated with different methods (ascorbic acid, citric acid, calcium chloride, sodium acid sulfate and infrared heat treatment) during storage at refrigerated conditions. The shorter storage period and low-temperature storage condition should be the reason for lower changes as compared to the present study as the higher storage temperature induces the decomposition of phenolic compounds. The decrease in total phenols could primarily result due to oxidative degradation of phenolic compounds and the polymerization of phenolic compounds with proteins [60]. The total reduction in TPC during 3 months storage was found to be 32.77 and 20.47 % in HW and OH treated samples, as a result of comparatively higher microbial growth during storage might have lead to the higher TPC degradation in HW treated samples. The nonthermal effects of OH on PPO inactivation (due to the presence of prosthetic metallic group 'Cu') would not have allowed the PPO to reactivate to that extent as that in HW may be another reason for higher degradation of TPC in HW treatment.



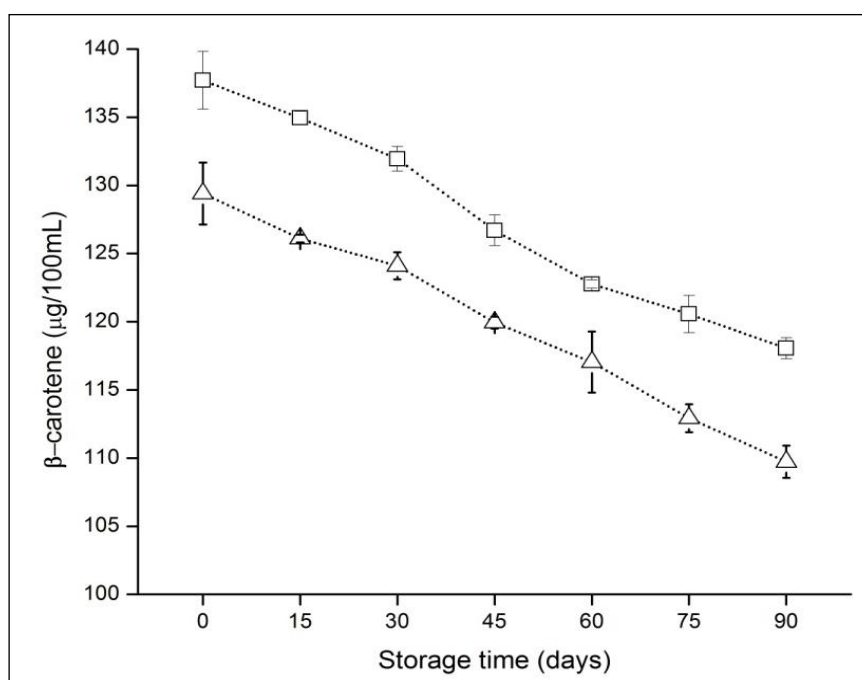
**Fig. 4.47** Change in the Total phenolic content of HW (□) and OH (Δ) treated mango puree sample during storage at ambient conditions

#### 4.5.7.5 Changes in $\beta$ -carotenes during storage

$\beta$ -carotenes are major coloring pigments in mango; the retention of  $\beta$ -carotene in food products is of substantial significance, as they are the primary sources of pro-vitamin A. Many studies have revealed that light, heat, oxygen, storage temperature, and interactions with packaging materials have a great role in the  $\beta$ -carotene degradation and

trans-cis isomerization [63, 81]. The  $\beta$ -carotenes were retained in higher amount during the HW treated as compared to OH treatment. During the storage, the  $\beta$ -carotenes were found to reduce significantly ( $p < 0.05$ ) in both HW and OH treated sample.

The reduction of 14.27 and 15.20 % was recorded in  $\beta$ -carotenes in HW and OH treated samples respectively during the storage of three months, the results are depicted in Fig. 4.47. Choi et al. [21] also reported a similar reduction in  $\beta$ -carotene during the storage of blood orange juice. These results infer that there was no difference in the rate of reduction in  $\beta$ -carotenes between the two treatments, but the difference in  $\beta$ -carotenes caused at the time of treatment was carried out straight away during the storage. The loss of  $\beta$ -carotene generally is due to auto-oxidation, the highly unsaturated chemical structure of  $\beta$ -carotenes makes them very susceptible to oxidation, and storage at ambient conditions enhances the rate of oxidation than at lower storage temperature [39].



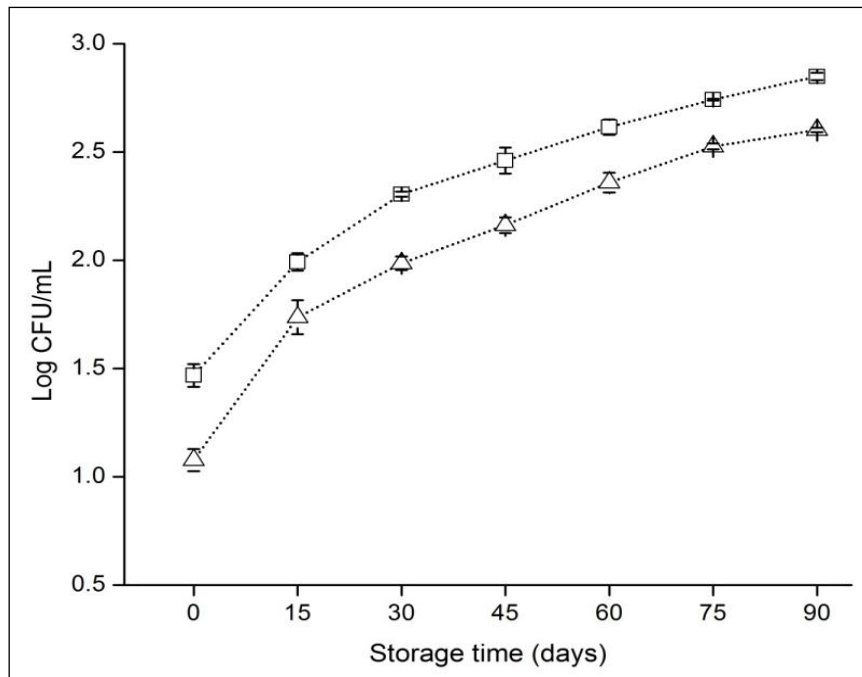
**Fig. 4.48 Change in  $\beta$ -carotenes of HW ( $\square$ ) and OH ( $\Delta$ ) treated mango puree sample during storage at ambient conditions**

As glass was used for the packaging, therefore, role of catalysis or promotion of auto-oxidation due to the metallic ion of packaging can be eliminated from the present study. Luc et al. [63] reported higher retention of  $\beta$ -carotenes (87.3 to 93.9 %) in thermally pasteurized mango puree; the higher retention might be due to the de-aeration of the product and or restriction of light-induced degradation of  $\beta$ -carotenes by using

opaque packaging materials for storing the product. The reduction in  $\beta$ -carotenes during the storage of six months was also reported in vacuum dried mango powder stored and packed in flexible packaging material; the loss was 30, 39 and 50 % after 2, 4 and 6-months of storage respectively [39]. On the contrary Provesi et al. [78] reported that no significant ( $P < 0.05$ ) change in concentration of carotenoids for six months storage of steam blanched and sterilized pumpkin puree, whereas the slight degree of isomerization of  $\beta$ -carotenes was caused by in blanching and sterilization. In present study some changes in  $\beta$ -carotenes was observed during 3 months storages, however the effect of treatment type was found insignificant for change in  $\beta$ -carotenes during storage, the change in  $\beta$ -carotenes in 3 months period was observed to be 14.27 and 15.20 % in HW and OH treated samples respectively. After a detailed study Lemmens et al. [58] recommended that the isomerization of  $\beta$ -carotenes is the function of the time of thermal treatment, whereas negligible isomerization was observed after high-pressure processing in mango puree.

#### 4.5.7.6 Changes in the Bacterial count during storage

Microbial safety is a great concern in food processing; therefore, many novel techniques are being studied to verify their feasibility to check the microbial growth during the storage. Ohmic heating can be used for microbial inactivation in food material to enhance its shelf life. The bacterial growth was observed to be very fast during the first one month of the storage whereas the rate of bacterial growth reduced after one month and onwards, which can be seen in Fig. 4.49. The reason for such finds may be due to the reduction in pH, as in acidic conditions the bacterial growth reduces. The bacterial count increased from 1.46 to 2.84 and 1.73 to 2.60 log CFU/mL in HW and OH treated samples. The pattern of increase in the BC was similar in both HW as well as OH treated samples, although there was an increase in the bacterial count the microbial population was below 6.0 log CFU/mL that was within an acceptable limit of the microbial count for fruit products for human consumption [51]. Guan et al. [32] reported that the high-pressure treatment reduced the plate count in mango juice significantly however the number of bacteria increased during the storage of two months under ambient condition. However, the growth was restricted to a greater extent by storage at refrigerated conditions.



**Fig. 4.49** Change in the bacterial count (BC) of HW (□) and OH (△) treated mango puree sample during storage at ambient conditions

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