



## Effect of Radio-Frequency Pre-Treatment on the Extraction of Tomato Oleoresin

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### ABSTRACT

*Tomato oleoresin represents the flavour of tomato used in natural flavourings and finds application as natural colouring agent also. The efficiency of wet extraction of tomato oleoresin using organic solvents is less due to the fact that carotenoids and other compounds are entrapped in tomato tissue. So the major challenge for the industries is to increase yield of thermolabile carotenoids in oleoresin which is having lot of health benefits. By taking into account the requirement the current study deals with effect non-thermal electromagnetic wave treatment (Radio-frequency) in extraction of tomato oleoresin. The solvent used is ethyl acetate and time of exposure is varied to study the effect exposure time of radiofrequency on oleoresin extraction. RF pre-treatment increased the yield of extraction. The lycopene yield was higher for 15 minutes pre-treated sample. SEM images of pre-treated sample's residue after extraction had fractures, which points to the effective rupture of tomato tissue which in turn facilitates the release of carotenoids. RF pre-treatment can be used as effective pre-treatment to increase the yield of tomato oleoresin when compared to the conventional extraction method.*

**Key words:** Carotenoids, Pre-treatment, Thermolabile, Non-thermal, Extraction.

### INTRODUCTION

Tomato flavour is a widely accepted flavour in the world, which is contributed by sugars, acids and volatiles present in it. The 16 compounds out of the identified 400 volatiles present are responsible for the flavour of tomato. Oleoresin contains these flavouring compounds except sugars and acids mainly aroma part<sup>1</sup>. The main advantage of tomato oleoresin is that it contains lycopene belonging

to the carotenoid family which imparts red colour and have many health benefits. Wet method of extraction is preferred over dry method as drying leads to loss of volatiles, imparts burned flavours and derivatives of flavour compounds are formed. But extraction of oleoresin from fresh tomato is very difficult because of its high water content which leads to the emulsification of solvent and water.

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So, low temperature drying techniques like freeze drying is used prior to solvent extraction. These are time consuming, costly process and volatile compounds are also lost during these processes. Thus proper pre-treatments are needed to increase the efficiency of extraction.

Lycopene is the major component of the oleoresin which exists mainly in trans-isomeric forms in plants and prone for degradation. It belongs to carotenoid family having lot of health benefits. It is an acyclic highly conjugated compound, can act as singlet oxygen scavenger which have anti-cancerous properties and DNA protection effect against oxidative stress<sup>2</sup>. Mostly solvents used for effective extraction of carotenoids are hexane and ethyl acetate<sup>3</sup>. Ethyl acetate, chosen for the study is produced from ethanol and acetic acid which can effectively extract  $\beta$  carotene and lutein effectively<sup>4</sup>. Ethyl acetate is a good grade solvent which gives safe extract for food applications. Extraction of tomato oleoresin using ethyl acetate is patented<sup>5</sup>. Yield of ethanolic extract of tomato is more than ethyl acetate extract even with 4.4 polarity index as lycopene is entrapped in plant matrix<sup>6</sup>. So, proper pre-treatment is required to disrupt cell structure to increase the efficiency of oleoresin extraction. For better diffusion of carotenoids from tomato tissue to solvent shorter and low temperature heat treatment is desirable. But longer and high temperature treatment can lead to deterioration of thermolabile carotenoids therefore development of non-thermal treatment for enhancing efficiency of extraction is necessary.

Microwave assisted extraction can reduce the time of extraction and increase the efficiency by releasing the lycopene from tomato tissues<sup>7</sup>. Microwave treatment can increase temperature of the system depending on dielectric behaviour of food produce and power level used. The radio frequency (RF) waves are having higher penetration than microwave because of its higher wavelength. Thus RF pre-treatment can be used as a pre-treatment for the better extraction of oleoresin

from tomatoes. Current study aims at development of non-thermal processing technology for extraction of tomato oleoresin.

## MATERIALS AND METHODS

### 1.1 Collection of raw materials

Tomatoes were procured from local market, Thajavur, Tamil Nadu, India. The chemicals used in this study are either lab grade or analytical grade, procured from Sigma-Aldrich.

### 1.2 Preparation of puree

Tomatoes were graded and over ripened were selected for further processing. Tomatoes were minced and homogenised in colloidal ball mill. The total soluble solid content of tomato puree was 4°Brix.

### 1.3 Extraction of oleoresin from tomato

100 g of tomato puree was macerated with 100 ml of ethyl acetate and filtered in muslin cloth. The ethyl acetate used for extraction contained 200 ppm of BHT (Butylated hydroxytoluene), a synthetic antioxidant to prevent degradation of lycopene during processing. The residue was again washed twice with same quantity of ethyl acetate. Then filtrate was transferred to a separating funnel to that 10 ml saturated sodium chloride solution was added to show better separation. The upper layer was collected and 100 ml of ethyl acetate was added to lower part to extract remaining lycopene. The middle layer that is solvent-water emulsion phase is collected, centrifuged at 3000 rpm for 10 minutes and upper layer was recanted. 500 mL of solvent used was for each 100 g of tomatoes puree. The Oleoresin obtained by this process was kept as control.

The effect of radio frequency pre-treatment was studied, by exposing tomato puree to RF radiations for different time intervals (15, 20, 25 mins). The power level was fixed from preliminary studies in such a way that the temperature of puree must not exceed  $50^{\circ} \pm 2^{\circ} \text{C}$  and by compensating time of exposure. The puree was poured to tray of 30×60 cm area to a height of 1cm above this height uniform heating was not happening. The power level was fixed from preliminary studies at 2500 W (40.68 MHz), below which longer time was taken to attain desired temperature. The

solvent extraction was done for all three pre-treated samples (15, 20, 25 mins) as mentioned earlier in the same section. The yield of oleoresins was noted. To know the effect of pre-treatment on colour of tomato puree, colour difference ( $\Delta E$ ) was calculated after RF treatment. The colour difference between residue and puree was noted to know the effectiveness of ethyl acetate in extracting colouring compounds of tomatoes like carotenoids.

#### 1.4 Analyses on extract

The carotenoid content of oleoresin was calculated spectrophotometrically using lycopene as standard<sup>8</sup>. The total polyphenol content of extract was calculated using Folin-Ciocalteu reagent by Spectrophotometric method<sup>9</sup>.

The Lycopene content of the same was confirmed by HPLC analysis (Shimadzu, promiece-i, LC-2030C 3D plus). The system was operated with C18 column, PDA detector, flow rate of mobile phase and column temperature was maintained at 1mL/minute and 40°C respectively. The Mobile phase used was acetonitrile 75%, methanol 20% and tetrahydrofuran 5%. The sampling speed was kept at 50 $\mu$ l/sec. Lycopene was used for plotting standard curve.

#### 1.5 Analyses on residue

The lycopene content of residue was analysed spectrophotometrically at 503nm<sup>10</sup>. The SEM image of residue was taken to analyse the effect RF treatment on structure of tomato cells with Tescan Vega 3 system. The images were taken after gold sputtering for 60secs (JEOL JFC -1600 Auto fie coater).

## RESULTS AND DISCUSSION

### 2.1 Changes in yield, carotenoid content, lycopene content and total phenolic content of tomato oleoresin

The moisture content of tomato puree was 93.62 $\pm$ .52 % on wet basis with lycopene content of 746.11 $\pm$ 0.46 mg/100g of dry matter. The yield, carotenoid content, lycopene content and total phenolic content of tomato oleoresin (ethyl acetate extract) is showed in table 1. The yield increased with pre-

treatment. But the yield of control, RF1 and RF3 showed non-significant difference. The reduced yield of RF3 may be due to the degradation of carotenoids and volatiles compounds with increased time of RF exposure. The time of exposure of RF didn't showed significant difference on yield. The control and RF3 showed non-significant difference. In control, carotenoids have trapped in tomato cell thus effective extraction cannot happened so reduction in carotenoid content have observed whereas in RF3 reduction is due to degradation of carotenoid with RF treatment. In RF1 and RF2 carotenoid content showed higher value as extraction will be effective with destruction of tomato cell by RF. This kind of tissue softening or destruction of tomato cell was reported in RF treated carrots also<sup>11</sup>. The highest total phenolic content is reported for RF3 which showed significant difference from all other samples in case of phenolic content. Thus we could conclude that RF pretreatment can be used in oleoresin extraction which has final application as natural antioxidant. The lycopene content was highest for RF1 followed by control. This shows enhanced extraction of lycopene from tomato tissue with pre-treatment. But with increase in time (more than 15 mins) of RF treatment lycopene degradation happens. The samples differ significantly with treatment time except for RF2 and RF3. Inference can be drawn from this results is more than 15 minutes of RF treatment is not advisable. The reduced content of lycopene with increase amount of carotenoid are observed in RF 2 and RF 3 this is because of formation of derivatives or degradation products from parent lycopene which is belonging to carotenoids or having same absorption peak of UV range.

### 2.2 Effect of Radio-frequency pre-treatment on the colour of tomato puree

The colour difference of RF treated puree with untreated puree is reported in table 2 to know the effect of treatment time on colour of sample. The colour difference of the samples were less than ten that means radio frequency treatment didn't changed the colour of the

puree to a notable range from untreated puree. We can conclude from statistical analysis that there is no significant difference between sample's colour difference. Thus the treatment is ideal and will not destruct the sample during processing. One of the main advantages of RF treatment is that it does not affect the colour while processing. This is important in maintaining the aesthetic appeal through visual appearance. Here it also indicates that neither lycopene degradation nor the sugar caramelisation happened. Similar acceptance was reported in sensory properties of RF treated ready to eat fresh carrots<sup>11</sup>.

### 2.3 Analyses on residue

The colour differences (with residue of untreated sample after extraction and RF pre-treated sample) showed in table 3 were less than 10 that means change in colour is not predominate. But with change in time of RF treatment the value differed significantly. RF3 was reported with highest colour difference which indicates that treatment helps in releasing lycopene from tomato matrix that is from pectineous material and improves efficiency of extraction. From this results it can be noted that time of exposure towards RF is having effect on extraction. The residual

lycopene content observed is noted in table 3 for all samples. Lycopene content in residue of untreated sample (control) is more than others and which differ significantly only from RF3. The reduced residual lycopene content points to increased efficiency of extraction. RF3 differs significantly from control. Orange colour of residual peel after microwave assisted extraction was observed<sup>7</sup>. This points that further processing has to be done in removing residual lycopene.

The SEM images shown in figure: 1, 2, 3 and 4 are images of residue after extraction with 2000X magnification. Figure 1 shows the residue's image of untreated tomato puree which didn't had fractures in the cells this may be the reason for reduced yield in the untreated sample. Figure 2, 3 and 4 had fractures which help in releasing the entrapped lycopene and other compounds in tomato. Fractures were formed as a result of RF pre-treatment. RF treatment will produce localised heat shots which in turn creates fractures in tissue that can enhance the efficiency of extraction. Similar kind of fissures are observed in residue after microwave assisted extraction<sup>7</sup>.

**Table 1: Change in yield, carotenoid content, total phenolic content and lycopene content of extract with RF treatment**

Pre-treatment	Time of RF treatment (min)	Yield(Percentage of dry matter of tomato)	Carotenoid Content of oleoresin ( mg /ml of extract)	Total phenolic content of (mg/100g of extract)	Lycopene content in extract (µg/g)
Control	0	1.14±0.13 <sup>a</sup>	96.74±6.3 <sup>a</sup>	19.98±1.33 <sup>a</sup>	13797.03±79.51 <sup>a</sup>
RF1	15	1.52±0.09 <sup>ab</sup>	136.90±3.89 <sup>b</sup>	23.40±1.40 <sup>ab</sup>	120235.50±509.62 <sup>b</sup>
RF2	20	1.79±0.21 <sup>b</sup>	134.18±8.28 <sup>b</sup>	26.09±2.17 <sup>b</sup>	3631.75±412.94 <sup>c</sup>
RF3	25	1.39±0.16 <sup>ab</sup>	93.62±10.01 <sup>a</sup>	33.24±4.33 <sup>c</sup>	3716.62±280.37 <sup>c</sup>

The data represented are mean ± standard deviation, The superscript with same alphabets didn't showed significant difference (P>0.05).

**Table 2: Colour difference with time of exposure of RF treatment**

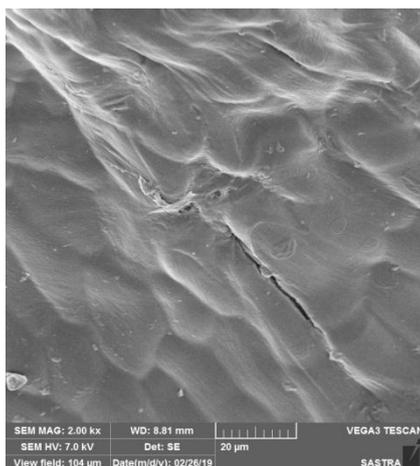
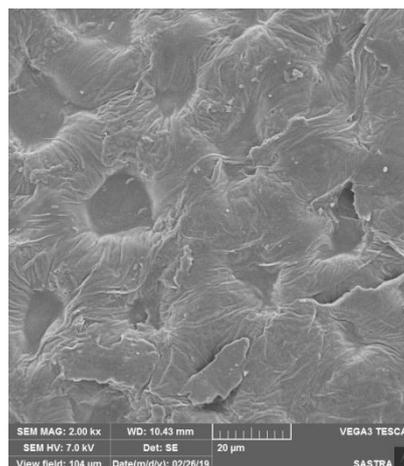
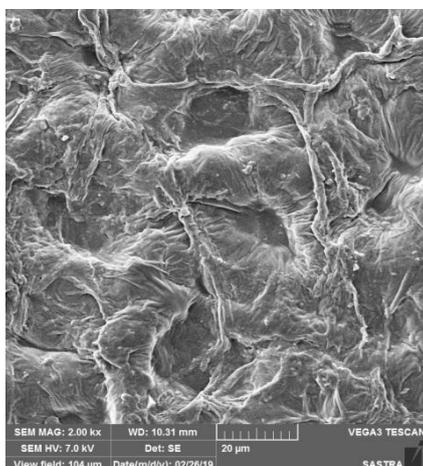
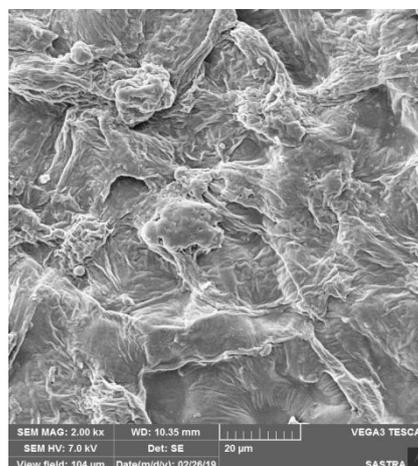
Treatment	Colour difference after RF treatment ( $\Delta E = \Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}$ )
RF1	0.81±0.15 <sup>a</sup>
RF2	4.54±0.06 <sup>a</sup>
RF3	2.64±0.15 <sup>a</sup>

The data represented are mean ± standard deviation, The superscript with same alphabets didn't showed significant difference (P>0.05).

**Table 3: Change in residual Lycopene content of residue after extraction with RF pre-treatment and colour difference of reissue with untreated sample residue**

Treatment	Colour difference of residue after extraction( $\Delta E$ ) compared to control sample	Lycopene content of residue after extraction (mg/100g of dry matter)
Control	***	4.90 $\pm$ 0.03 <sup>a</sup>
RF1	2.17 $\pm$ 0.29 <sup>a</sup>	4.25 $\pm$ 0.50 <sup>ab</sup>
RF2	2.58 $\pm$ 0.20 <sup>b</sup>	4.04 $\pm$ 0.05 <sup>ab</sup>
RF3	2.75 $\pm$ 0.14 <sup>c</sup>	3.65 $\pm$ 0.12 <sup>b</sup>

The data represented are mean  $\pm$  standard deviation, The superscript with same alphabets didn't showed significant difference ( $P > 0.05$ ).

**Figure 1: Untreated Sample****Figure 2: 15 minutes RF pre-treatment****Figure 3: 20 minutes RF pre-treatment****Figure 4: 25 minutes RF pre-treatment**

### CONCLUSION

From this study it is concluded that RF pre-treatment can be used for enhancing the efficiency of extraction of oleoresin from high moisture produce (wet extraction). The time of treatment and power level will vary with moisture content of produce and tissue hardness. The 15 minutes of pre-treatment is ideal for preparation of lycopene rich oleoresin. Whereas yield is highest for sample with 20 minutes pre-treatment. The RF pre-

treatment increased the amount of bioactive compounds in the extract that means the carotenoid content and colour of tomato puree was not affected by RF treatment which points this non-thermal technology as an ideal pre-treatment for extraction colouring compounds. The pre-treatment must be optimised by taking final product application into account. These oleoresins can represent complete flavour of the fruit thus have applications in flavouring, wine, bakery and snack industry.

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