

Pulsed electric field and combination processing of mango nectar: effect on volatile compounds and HMF formation

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Summary

Mango nectar is a commercially familiar and preferred product. The traditional processing of mango nectar has been by thermal processing which resulted in the alteration of the flavour of the product due to the effect of high temperature. The thermal processing of the nectar also resulted in the production of byproducts of non-enzymatic browning such as 5-hydroxy methyl furfural (HMF). These process induced effects, affect both the nutritive and sensory attributes of the fruit product, making it less preferable. With the growing interest and awareness about the benefits of alternative non-thermal technologies, such as pulsed electric field (PEF), the present work was proposed to use PEF to minimize the loss of volatiles and formation of HMF. The study involves thermal (96 °C for 300 s and 600 s), PEF (24 μs, 120 Hz and 38 kV/cm) and combination processing (PEF + Thermal (96 °C for 90 s)) of mango nectar. The effect of these treatments on the volatile composition of mango nectar has been analysed using GC-MS technique. The reduction in the volatile compounds was significant ($p < 0.05$) in the samples treated by thermal and combination processing. The HPLC determination of HMF was observed to increase with storage and also with thermal treatment. Overall, the study showed that PEF treatment had less negative effect on the retention of volatile compounds and also the HMF concentration was on the lower side compared to the other treated samples and was insignificantly ($p > 0.05$) different from unprocessed sample, proving the fresh-like character of the product.

Keywords: mango nectar, pasteurization, pulsed electric field (PEF), HPLC, GC-MS, HMF, flavor, volatile compounds

Introduction

Mango (*Mangifera indica* L.) is one of the most important and popular tropical fruits that originates from Southeast Asia and has been cultivated for at least 4,000 years (Santhirasegaram et al., 2013). Known for its vibrant colours, exotic flavours, distinctive taste, and nutritional properties, it is of high economic importance in the international market (Singh et al., 2013). The fruits from the several hundred known cultivars differ greatly in their flavour characteristics. More than 270 volatile compounds that contribute to the aroma of fresh and processed mangoes have been identified and extensively studied in many cultivars (Shibamoto and Tang, 1990; TNO, 1996; Winterhalter, 1991). The volatiles of mango cultivars were observed to comprise monoterpene and sesquiterpene hydrocarbons, the most abundant mango volatiles (Lalel et al., 2003; Andrade et al., 2000); whereas the esters and lactones play a vital part in imparting the unique flavour of certain cultivars. Mango nectar is one of the most preferred mango products, which is produced by mixing the puree with citric acid, sugar and water to a proscribed Brix/acid ratio, dependent upon raw material and desired use.

Conventional heat treatment is the most common and preferred technology used to preserve seasonal and perishable fruits and vegetables, thereby making them available round the year and around the world. Thermal processing is performed with the objective to prevent microbial spoilage, enzymatic browning and also to extend its shelf life. During processing the product undergoes physical and chemical changes that impair the organoleptic properties and also may reduce the content or bioavailability of some bioactive compounds (Rawson et al., 2011). In the processed fruit products, accumulation of brown colour during processing occurs due to enzymatic and non-enzymatic reactions. These non-enzymatic reactions involve caramelization, ascorbic acid degradation and Maillard reaction. While caramelization occurs on heat treatment of sugars at high temperatures, ascorbic acid degradation occurs by an oxidative path in citrus juices. The Maillard reaction, taking place between α -amino groups and reducing sugars, is the most important cause of browning in the juice. The Maillard reaction also produces volatile and non-volatile flavour compounds such as furans, furanones, furaneol, sotolone, etc.

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During processing and storage, the Maillard reaction, generates different furfural derivatives, leading to desirable or undesirable changes in the food quality wherein the aroma, taste, colour and nutritional value of the food are altered. 5-Hydroxymethylfurfural (5-HMF), an aldehyde is a characteristic intermediate flavour compound produced during the primary stages of the Maillard reaction between hexoses and α -amino acids, considered as an indication of quality deterioration, which is practically not present in fresh food (Cioroi, 2008). Hydroxymethylfurfural (HMF) is also considered to an indicator of quality deterioration as a result of excessive heating or storage in a wide range of food containing carbohydrates and amino acids/ proteins (Cioroi, 2008). HMF formation in a product depends on factors such as sugar concentration, acidity of the product, ascorbic acid content, temperature of treatment and storage as well, heating time and also depends on storage condition. Concentration of 5-HMF also increases during the heating or storage processes (Lansalot-Matras and Moreau, 2003), and it is commonly found in honey, fruit juices, UHT milk, coffee and dried fruit. The International Federation of Fruit Juice Processors (IFFJP) has recommended maximum concentrations of 5-10 mg/l and 25 mg/kg in fruit juices and concentrates, respectively (Wagner and Beil-Seidler, 2006); and the European Union has set a limit of 20 mg/kg 5-HMF for juices made for children (FPA, 2006).

The processing of the fruit juices not only alters the flavour of the product, but also produces certain characteristic flavour during both processing and storage. The volatile and non-volatile reaction products are also generated as a the result of chemical interaction of the hexoses, α -amino acids, peptides, proteins, biogene amines, vitamins with amino groups and others (Cioroi, 2008). The characteristic volatiles (including alcohols, aldehydes, furans, ketones, terpenes, and others) are often involved in process-induced reactions (e.g., Maillard reaction, Strecker degradation, oxidative degradation of unsaturated fatty acids and carotenoids) (Bedair et al., 2008) which can be used as specific marker compounds, as it is linked to the possible reaction pathways or particular food characteristics in different conditions (Dettmer et al., 2007; Kebede et al., 2013a; Vervoort et al., 2013; Wishart, 2008). The headspace volatiles of samples are widely measured by Gas chromatography-Mass spectrometry (GC-MS), which has been proven to be effective for an unbiased comparison of quality changes occurring through the processing chain, i.e. due to different food processing, preservation, and subsequent storage condition.

The growing interest in mildly processed products has facilitated the development of novel non-thermal food

preservation methods (e.g., high hydrostatic pressure, pulsed electric field processing, high pressure carbon dioxide). Pulsed electric field processing (PEF) is one of these alternative novel technologies which have the potential to strike the balance between safety and quality characteristics of food products. The studies established until recently, have suggested that PEF treatment is efficient enough to destroy microorganisms in the fruit juices at levels equivalent to those achieved by heat pasteurisation without greatly affecting their nutritional and sensory properties (Yeom et al., 2000; Min and Zhang, 2003). In addition, the enzymes commonly present in fruit juices are partially or totally inactivated by PEF treatment (Marselles-Fontanet and Martin-Belloso, 2007).

Unfortunately, juice flavour compounds are very unstable during processing and storage (Braddock, 1999). There have been reports that show significant quality losses in thermally treated mango fruit (Kim et al., 2009), mango slices (Chen et al., 2007), and mango nectar (Vasquez-Caicedo et al., 2007). Chemical compounds responsible for the colour and flavour of fruit juices are retained in a higher ratio, when compared with fresh, untreated samples, by the use of non-thermal pasteurization techniques such as pulsed electric fields (Aguilar-Rosas et al., 2007). Thus, the increasing demand for fresh-like juices has led to a growing interest in non-thermal processing technologies such as pulsed electric fields (PEF). On the other hand, little is known about the evolution of flavour and other compounds such as HMF in PEF-processed juices. Therefore, the aim of this research was to study and compare the effects of PEF processing, heat treatments and combination treatments on the flavour compounds of mango nectar.

Materials and methods

Pre-processing steps

Ripe mangoes of Mallika variety of desired quality were purchased at a local market in Mysore, India. The mangoes were sorted, cleaned and washed before taken for the nectar preparation. The washed mangoes were then de-skinned, de-stoned, sliced and finally pulped using a pulper. The pH of the Ready-to-Drink (RTD) mango nectar was adjusted to 3.5 ± 0.01 with citric acid, and the total soluble solid was adjusted to $18.0 \pm 0.10^\circ\text{Bx}$ with sucrose. Since headspace volatile changes of mango nectar may be attributed to enzymatic and chemical reactions during pre and post- processing, utmost care was taken to keep the changes in initial volatile compounds minimal during preliminary steps.

Processing

The RTD mango nectar was divided into five parts, which were subjected to treatments, such as in-pack pasteurization, Pulsed Electric Field Treatment and combination processing which were performed in triplicates and the average of the values were determined and used for data analysis. The same multilayered (four layered) pouches were used for packing all the samples.

Thermal processing

Two parts of the RTD mango nectar were packed in 4-layer laminated pouches (comprising of (outer to inner layer): polyethylene, oriented polyamide, aluminium and polypropylene) of 200 ml volume in each pack and sealed hermetically by an impulse heat sealer (Model: HP Impulse Sealer, M/s Sunray Industries Mysore, India), after manually removing the entrapped air from the head space of the pouch, by flushing in with steam. The sealing of the pouches were carried out at a sealing temperature of 200 °C, dwell time 10 secs and air pressure of 4.5 kg/cm². The packed and sealed pouches were subjected to in-pack pasteurization in a steam jacketed kettle using a steel basket with proper closure. The temperature of the product and the kettle was continuously recorded during processing by thermo-electro motive force at regular intervals with the help of copper-constantan thermocouples, which were fixed at the geometric centre of the pouches and retort. Thermocouple output was measured using an Ellab CTF 9008 data recorder (Ellab A/S, Roedovre, Denmark). The packed nectar was treated at 96 °C for 300 s (T2) and at 96 °C for 600 s (T3) respectively. The processed samples were immediately cooled by immersing in cold running water to curb reaction initiated by processing. The samples were then analyzed for the required parameters.

Pulsed Electric Field processing

Pulsed Electric Field (PEF) treatments were performed using a pilot scale continuous PEF system (Model: ELCRACK® HVP 5, DIL, German Institute of Food Technologies, Quackenbruck, Germany) with bipolar square-wave pulses through an electrode gap of 10 mm. The maximum voltage was 80 kV, the maximum frequency was 1 kHz and the pulse width was adjustable between 4 and 32 µs. The system consisted of co-linear treatment chambers followed by an AKG-cooling system (-5 °C), which was also connected with the PEF equipment during the processing of the samples. The characteristics of the

electric pulses delivered such as shape, polarity, width, difference of potential as well as the electric current generated across the electrodes and the pulse frequency were monitored using a digital oscilloscope (Model: Digital touch screen oscilloscope Siemens, Made in Denmark). Temperatures were monitored by two thermocouples (Testo AG, Lenzkirch, Germany) with a pipe wrap type probe attached to the surface of the stainless-steel tubes at the inlet and outlet points of the unit. The final recorded temperature of the product did not exceed 35 °C, where a maximum increase of 5 °C was observed from the initial sample temperature (30 °C). The temperature variation was also monitored and measure using the Ellab digital data recorder (Model: Ellab CTF 9008, Make: Ellab A/S, Roedovre, Denmark). The RTD mango nectar was pumped through the system using a peristaltic pump (Type SK 20F-80 L 14 TF T 10/ 1-S. Getriebefow Nord Bargetheid, Germany) at a flow rate of 41 l/hr. The PEF treatment was optimized for its process parameters (frequency and pulse width), wherein the frequencies were varied between 70 and 120 Hz and the pulse width ranged from 15 µs to 24 µs using bipolar mode pulses of constant field strength 38 kV/cm, for all the treatments (Kumar et al., 2015). 200 ml of processed samples from each batch were filled in sterile (thermally) pre-fabricated multilayer laminated pouches consisting of 12 µm Polyethylene terephthalate / 9 µm Aluminium foil / 15 µm Nylon / 80 µm Cast. Polypropylene (Total thickness 116 µm) pouches with a dimension of 15 X 20 cm under sterile conditions and hermetically sealed using impulse sealing machine (Model: HP Impulse Sealer, M/s Sunray Industries Mysore, India). The experiments were performed in triplicate.

Combination processing

The RTD mango nectar was subjected to a combination of treatments such as PEF followed by thermal processing. The nectar was first subjected to the PEF treatment with pulse parameters set as 24 µs pulse width, 120 Hz frequency and 38 kV/cm electric field strength; flow rate of 45 l/hr. Then the packed PEF processed sample was thermally treated by in-pack pasteurization method at 96 °C for 90 s. The treated sample (T5) was cooled immediately after treatment and stored for storage study.

Post-processing

Aliquots were drawn as needed for analysis from the processed samples, titled as T1 (Control), T2 (Pasteurization for 300 s), T3 (Pasteurization for 600 s),

T4 (PEF) and T5 (PEF+ Pasteurization for 90s). Three replicates were used for each nectar.

Gas Chromatography- Mass Spectrometry (GC-MS) analysis of volatile compounds

Extract for the analysis was prepared by simultaneous distillation-extraction of volatiles using diethyl ether (25 ml) for 2 h from the mixture of homogenized sample (1:3 ratio of sample and distilled water) and 0.1 g of methyl nonanoate (used as internal standard). One μl of the extract was injected for GC- MS analysis (Pino et al., 2005).

GC- MS analysis was run on Agilent (Model; 6890 N) gas chromatograph interfaced with an Agilent 5973 inert Mass detector. A HP-5 MS capillary column (30 m x 0.25 mm x 0.25 m) was employed for the separation with cross bonded diphenyl (5%) and dimethyl polysiloxane (95%) as a stationary phase. The oven was programmed at 50 °C for 2 min and ramped to 10 °C /min up to 250 °C after which it was held at this temperature for 5 min. The GC injector temperature was set at 250 °C with the injector split ratio set to 50:1. Helium was used as a carrier gas with a constant flow rate of 1.0 ml /min. The separated compounds from the GC were transferred to mass spectrometer via a heated interface maintained at 280 °C. The ion source and quadruple mass analyzer temperatures were kept at 220 °C and 150 °C respectively. Solvent delay was fixed to be 3 min. The mass spectrometer was operated at 70eV in EI mode with the mass range of 50-550 am. Volatile compounds present in the mango nectar samples were identified by using NIST library search.

HPLC Determination of 5-Hydroxy Methyl Furfural (HMF)

The HMF analysis was performed for samples immediately after processing and also for the samples stored at ambient condition (25-30 °C) for a period of 90 days.

An aliquot of 200 ml sample was diluted to a volume of 500 ml with MilliQ-water and homogenized. The diluted solution was centrifuged at 4000 rpm for 10 min. 5 ml of the supernatant was taken and filtered through 0.45 μm membrane filter. 20 μL of this filtrate was used for each injection to HPLC. The chromatographic system consisted of a Waters 515 pump; a Waters Model 2489 UV-visible detector; a manual injector and Digital data station. Separations were carried out on a 250 x 4.6 mm i.d. column packed with 5 μm Spherisorb ODS-2. The mobile phase was acetonitrile/water (8:92) at 1 ml/min,

degassed with a sonicator prior to use. The analysis was carried out by injecting 20 μl of the sample or standard into the column. Final UV detection was carried out at 280 nm. Standard solutions of HMF (Sigma Chemicals) of concentration 250 ppb to 2500 ppb were prepared by dissolving in Milli-Q water (Method of Balanco et al. (1991) with a few modifications).

Sensory quality

Sensory quality was determined using 9 point Hedonic scale according to Ranganna, (1999). For sensory taste and odor evaluation, 20 semi trained panelists were selected. The 100ml samples were presented to the panelists, who rated the preferred samples in comparison with the untreated control sample, for over all acceptability (OAA).

Statistical analysis

Data were analyzed by the least-squares method and response surfaces were generated using the Design Expert 7.0.0 software (Stat Ease Inc., Minneapolis, MN). Analysis of variance (ANOVA) was used to test the significance of each variable ($\alpha = 0.05$) and to verify the adequacy of the model. Interaction effects were determined using LS means ($\alpha = 0.05$). All assays were carried out in triplicate.

The experiments were conducted in three replicates and Student Newman Keuls test was performed to determine the statistical difference at $p < 0.05$ with the help of ANOVA. (Coplots: 2003. CoStat Version 6.204)

Results and discussion

The samples were subjected to various treatments and labelled for convenience of analysis. The PEF process of the RTD mango nectar was optimized and the optimized input parameters were fed into the PEF process system and the operational out-put process parameters such as flow rate, pulse field strength, energy, frequency and load resistance were calculated and presented in the Table 1 (Kumar et al., 2015). The maximum of 38.0 kV/cm pulse field strength was achieved during the process of the sample, which was numbered has T4 and T5 (combination treatment). Pasteurization of the mango nectar was performed at a product temperature of 96 °C at varying treatment time and marked as T2 (300 s), T3 (600 s) and T5 (combination treatment for 90 s) respectively. Total heating time (*f_h*) and *P value* of the thermal treatments T2 and T3 was calculated and presented in the Table 2.

Table 1. Optimized PEF processing parameters

Parameters	Units	Values	Parameters	Units	Values
In-put Parameters			Out-put Parameters		
Output Voltage	[%]	60	Flow rate	[ltr/hr]	41.2
Pulse width	[μ s]	24	Pulse Field Strength	[kV/Cm]	38.0
Frequency	[Hz]	120	Energy	[KJ/ltr]	219.3
Flow rate	[ltr/hr]	45.0	Frequency	[Hz]	108.0
Electrode Gap	[mm]	10	Load Resistance	[ohm Ω]	113

(Source: Kumar et al., 2015)

Table 2. Thermal Processing parameters and *P value*

Parameters	T2	T3
Temperature	96 °C	96 °C
Total heating time (<i>fh</i>)	300 s	600 s
<i>P value</i>	1.2	8.03

The volatile compounds of unprocessed (T1) and processed (T2, T3, T4 and T5) were represented in the Table 3 and Fig. 1. Monoterpene hydrocarbons quantitatively represent the main group of volatile compounds in mango as indicated by Andrade et al. (2000) and Lalel (2003). (Z)-Ocimene compound was found to be abundant in the volatile composition of all the samples. The (Z)-Ocimene compound found to be significantly ($p < 0.05$) reduced between the unprocessed ($160 \pm 0.520 \mu\text{g g}^{-1}$) and processed mango nectar (ranging from 150-159 $\mu\text{g g}^{-1}$). Mesifuran is the other important compound, which seconds the list of compound based on the concentration and was found to be $12.30 \mu\text{g g}^{-1}$ in T1 sample. It was also found to decrease significantly ($p < 0.05$) between the processed sample. The other volatile compounds identified and evaluated were (E)-Ocimene, γ -Octalactone, δ -Hexalactone, β -Caryophyllene, γ -Hexalactone, γ -Butyrolactone, Longifolene, *Allo*-ocimene, δ -Octalactone, Humulene, Longicyclene, γ -Decalactone, Isolongifolene, Limonene, Myrcene and α -Pinene, were observed to be in minor quantity. These minor volatile compounds were also found to be affected significantly ($p < 0.05$) by the effect of processing. Our results were in accordance with the results of other authors such as El-Nemr et al. (1988), Min and Zhang (2003), Jorge and Judith (2006), Vásquez-Caicedo et al. (2007) and Aguiló-Aguayo et al. (2010). El-Nemr et al. (1988) observed a decrease in concentration of all volatile fractions in mango nectar with processing, and they attributed this fact to evaporation, specially the esters fractions. According to Buttery et al. (1990) and Rousef and Leahy (1995), high temperatures during

processing can greatly affect the flavour profile of juice products. There have been reports that show significant quality losses in thermally treated mango nectar (Vásquez-Caicedo et al., 2007). Aguilar-Rosas et al. (2007) reported that the concentration of volatile compounds in an apple juice was less affected by PEF treatments than by thermal pasteurization. In the study by Aguiló-Aguayo et al. (2010), the PEF processing of watermelon juice, not only induced a rise (roughly 20%) in the concentrations of some volatile flavour compounds, but also achieved less reductions on the retention of volatiles than the thermal treatment. The higher retention of volatiles in PEF processed samples was due to the effect of lower processing temperature, i.e. the maximum temperature achieved at the PEF processing was 35 °C. This was in agreement with Min and Zhang (2003) and Aguiló-Aguayo et al. (2010), who reported that the lower processing temperatures, induced on samples by PEF processing, may favour flavour retention in PEF-treated juices. In the case of combination treatment, the loss of volatile is more when compared to all the treatments, primarily due to the effect of thermal treatment. This was consistent with the findings of Sukan and Russell (2002), who reported that the loss of aroma-related volatile compounds in juices could be induced by volatilisation during heat processing. It was also observed that the volatile compound concentration was decreased insignificantly ($p > 0.05$) in the PEF treated sample (T4) when compared to the control sample (T1). The T1 and T4 values were observed to differ significantly ($p < 0.05$) from the values obtained from the T2, T3 and T5 sample.

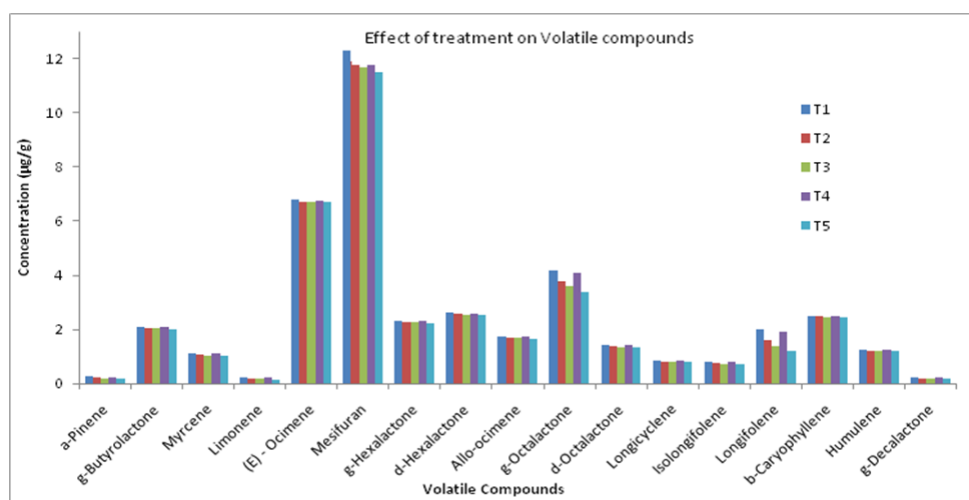
Table 3. Effect of thermal, PEF and combination (PEF + Heat) processing on volatile composition ($\mu\text{g g}^{-1}$) of mango nectar

Compounds	Treatments				
	T1	T2	T3	T4	T5
α -Pinene ¹	0.26 \pm 0.006 ^a	0.22 \pm 0.000 ^c	0.20 \pm 0.006 ^d	0.25 \pm 0.006 ^b	0.18 \pm 0.000 ^e
γ -Butyrolactone	2.10 \pm 0.015 ^a	2.06 \pm 0.006 ^{abc}	2.03 \pm 0.006 ^{bc}	2.09 \pm 0.006 ^{ab}	2.02 \pm 0.006 ^c
Myrcene ¹	1.11 \pm 0.000 ^a	1.07 \pm 0.000 ^b	1.05 \pm 0.006 ^c	1.11 \pm 0.010 ^a	1.03 \pm 0.006 ^d
Limonene ¹	0.24 \pm 0.006 ^a	0.20 \pm 0.015 ^b	0.18 \pm 0.000 ^c	0.23 \pm 0.006 ^a	0.16 \pm 0.000 ^d
(Z) - Ocimene	160 \pm 0.520 ^a	156 \pm 0.000 ^c	154 \pm 0.058 ^d	159.03 \pm .058 ^b	152.0 \pm 0.000 ^e
(E) - Ocimene	6.78 \pm 0.012 ^a	6.72 \pm 0.010 ^b	6.72 \pm 0.006 ^b	6.76 \pm 0.000 ^a	6.71 \pm 0.010 ^b
Mesifuran ¹	12.30 \pm 0.000 ^a	11.90 \pm 0.000 ^c	11.70 \pm 0.015 ^d	12.20 \pm 0.000 ^b	11.50 \pm 0.010 ^e
γ -Hexalactone ¹	2.31 \pm 0.010 ^a	2.27 \pm 0.006 ^b	2.26 \pm 0.000 ^c	2.31 \pm 0.006 ^a	2.23 \pm 0.012 ^d
δ -Hexalactone ¹	2.61 \pm 0.000 ^a	2.57 \pm 0.006 ^b	2.55 \pm 0.006 ^c	2.60 \pm 0.006 ^a	2.53 \pm 0.000 ^d
Allo-ocimene	1.75 \pm 0.006 ^a	1.71 \pm 0.020 ^b	1.69 \pm 0.000 ^b	1.74 \pm 0.020 ^a	1.66 \pm 0.006 ^c
γ -Octalactone ¹	4.20 \pm 0.015 ^a	3.77 \pm 0.058 ^c	3.60 \pm 0.006 ^d	4.10 \pm 0.000 ^b	3.40 \pm 0.000 ^e
δ -Octalactone	1.43 \pm 0.012 ^a	1.38 \pm 0.000 ^b	1.36 \pm 0.010 ^c	1.41 \pm 0.006 ^a	1.34 \pm 0.006 ^{cd}
Longicyclene	0.87 \pm 0.006 ^a	0.83 \pm 0.006 ^c	0.81 \pm 0.000 ^d	0.86 \pm 0.006 ^b	0.79 \pm 0.010 ^e
Isolongifolene ¹	0.79 \pm 0.006 ^a	0.76 \pm 0.000 ^b	0.73 \pm 0.006 ^a	0.79 \pm 0.006 ^a	0.72 \pm 0.006 ^c
Longifolene ¹	2.00 \pm 0.000 ^a	1.61 \pm 0.010 ^c	1.40 \pm 0.000 ^d	1.90 \pm 0.000 ^b	1.23 \pm 0.058 ^e
β -Caryophyllene ¹	2.51 \pm 0.006 ^a	2.51 \pm 0.064 ^a	2.45 \pm 0.006 ^{ab}	2.50 \pm 0.010 ^a	2.44 \pm 0.010 ^{ab}
Humulene ¹	1.27 \pm 0.006 ^a	1.23 \pm 0.006 ^b	1.23 \pm 0.015 ^b	1.27 \pm 0.000 ^a	1.20 \pm 0.000 ^c
γ -Decalactone ¹	0.24 \pm 0.021 ^a	0.21 \pm 0.006 ^b	0.19 \pm 0.000 ^c	0.24 \pm 0.006 ^a	0.17 \pm 0.006 ^{cd}

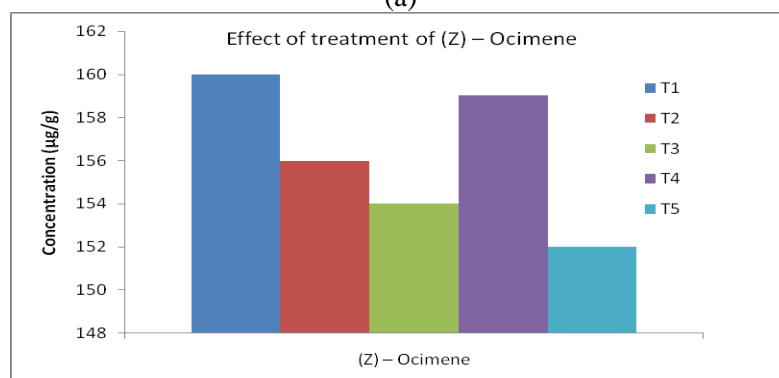
Means with the SD superscripts in a column vary significantly ($p < 0.05$)

¹These Compounds were identified by comparing their Mass spectrum and Kovat's index with those of authentic external; the rest of the compounds were identified by comparing their mass spectrum and Kovat's index with those reported in the literature.

T1= Control, T2= Pasteurization for 300 s, T 3= Pasteurization for 600 s, T4= PEF, T5= PEF+ Pasteurization for 90 s



(a)



(b)

Fig. 1. The effect of different treatment on the volatile composition of mango nectar

One of the degradation products of sugars during the Maillard reaction, HMF was the important quality index of fruit juices, as it was rich in hexose and ketose sugar which were prone to degradation. Due to heat treatment and increased dry matter juice concentrates, it was reported to be more susceptible to non-enzymatic browning. The HMF standards were injected at various concentrations to find out the calibration curve. The calibration curve for HMF standards was presented in the Fig. 2. The responses of each standard peak area, peak height were presented in the Table 4. The R^2 of the calibration curve was found to be 0.999995. The HMF concentration in the samples (T1, T2, T3, T4 and T5) was analyzed before and after storage by HPLC technique and the values were presented in the Table 5. Fig. 3 represents the chromatogram for the sample T3 after storage. The control sample (T1) 5-Hydroxy Methyl Furfural (HMF) was found to be 52.5 ppb. After the processing, the samples of T2, T3, T4 and T5 were found to have a concentration of 186.23 ppb, 254.2 ppb,

80.23ppb and 95.32ppb respectively. Here in the T3 sample, it is found to be higher when compared to other processing samples, due to the application of heat for a longer time period (600 s). The concentration of HMF in samples T2, T3, T4 and T5 were found to have increased to a value 382.32 ppb, 450.247 ppb, 120.32 ppb and 145.3 ppb respectively, by the end of the storage study. The increase in the concentration of HMF was higher in the thermally treated samples, which was dependent on the time of exposure. On comparing the T4 and T5 samples, its values also followed a same trend as that of the thermal treatment, which corresponds to the increase in value, since HMF is an autocatalytic reaction and its initial concentration at the beginning of a storage period may have its influence in further increase during storage. The value in T5 was observed to be slightly higher than T4, due to combination of PEF and Thermal treatment, but was significantly lower than T2 and T3 because of the short time exposure (60 °C).

Table 4. 5-Hydroxy Methyl Furfural (HMF) standards and responses

S.No	Sample Name	Result ID	Peak Name	X Value	Responses	Calc. Value	% Deviation
1	HMF 250 ppb	1547	HMF-1	250.00	61405.850	250.664	0.27
2	HMF 500 ppb	1550	HMF-2	500.00	127207.375	498.352	-0.33
3	HMF 1000 ppb	1551	HMF-3	1000.00	260508.132	1000.119	0.01
4	HMF 2000 ppb	1552	HMF-4	2000.00	527002.956	2003.250	0.16
5	HMF 2500 ppb	1553	HMF-5	2500.00	658337.379	2497.616	-0.10

Table 5. Effect of thermal, PEF and combination (PEF + Heat) processing on 5-Hydroxy Methyl Furfural (HMF) content

Parameters	T1	T2	T3	T4	T5
HMF content after processing (ppb)	52.5 ± 0.25	186.22 ± 0.08	254.21 ± 0.18	80.25 ± 0.20	95.32 ± 0.26
HMF content after storage period (90 days) (ppb)		382.32 ± 0.27	450.25 ± 0.41	120.32 ± 0.18	145.3 ± 0.25

Mean±SD

T1= Control, T2= Pasteurization for 300 s, T3= Pasteurization for 600 s, T4= PEF, T5= PEF+ Pasteurization for 90 s

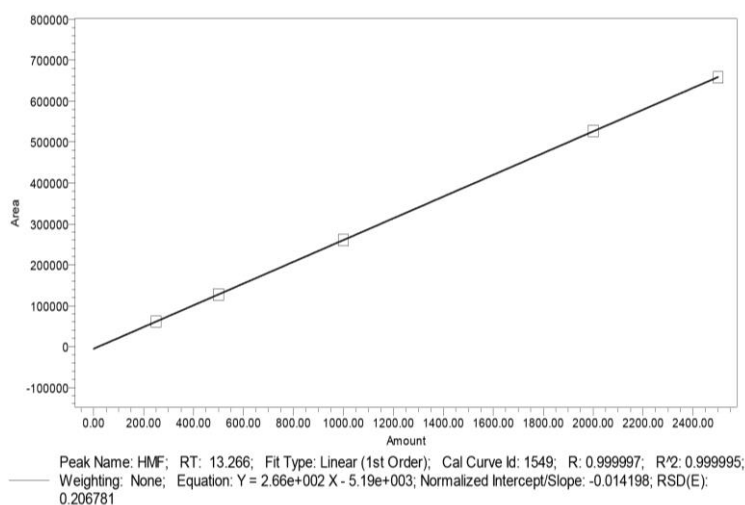


Fig 2. Calibration curve for standards HMF standards (250 ppb to 2500 ppb)

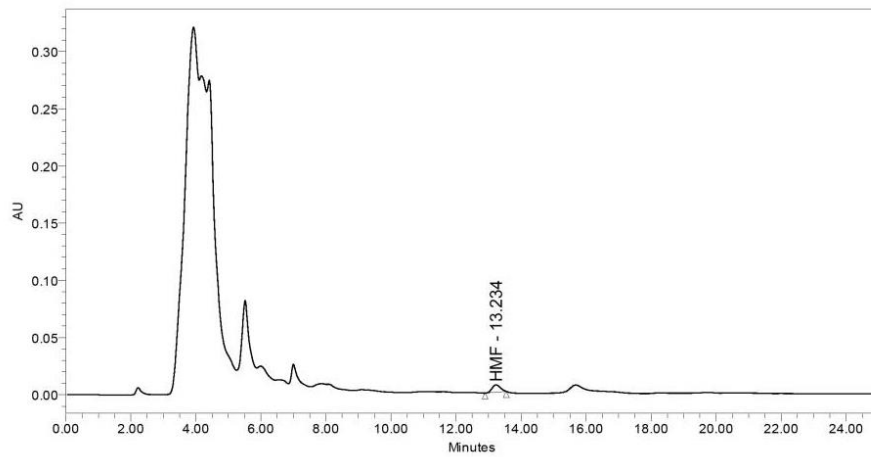


Fig 3. HPLC chromatograms for T₃ sample after storage

The sensory score was based on the 9 point hedonic scale rating given by the panelist, the quality attributes of color, appearance, consistency, flavour and overall acceptability (OAA) was taken. Table 6 represents the 9 point hedonic scale rating given for the untreated and processed mango nectar. The untreated (T1) mango nectar sensory score was found to be 8.5 ± 0.100 , 8.6 ± 0.000 , 8.3 ± 0.058 , 8.7 ± 0.058 and 8.5 ± 0.000 for color, appearance, consistency, flavour and overall acceptability (OAA) respectively. The PEF (T4), PEF + pasteurization (T5) samples were found to be high rating when compare to the samples processed by

thermal pasteurization (T2 and T3). The sensory scores obtained by the PEF treated sample were found to retain the fresh like characteristics as in the untreated sample (Kumar et al., 2015). The samples stored at room temperature was found to be significantly ($p < 0.05$) reduced throughout the storage period. Min and Zhang (2003) also found that thermally processed juice had a significant decreased in the sensory score. Overall, it could be stated that PEF treatment can be used for fruit products to reduce the HMF formation or accumulation and to increase the retention of flavour compounds.

Table 6. Effect of thermal, PEF and combination (PEF + Heat) processing on sensory qualities of mango nectar during storage at room temperature

Quality attributes	Storage Period (Days)	Treatments				
		T1	T2	T3	T4	T5
Colour	0	8.5 ± 0.058^a	8.1 ± 0.000^d	8.0 ± 0.058^c	8.2 ± 0.000^c	8.4 ± 0.000^a
Appearance		8.6 ± 0.100^{ab}	8.1 ± 0.200^{cd}	8.2 ± 0.058^d	8.6 ± 0.058^a	8.1 ± 0.100^{cd}
Consistency		8.3 ± 0.058^{bc}	8.2 ± 0.153^{bc}	8.2 ± 0.153^c	8.4 ± 0.058^{bc}	8.5 ± 0.058^{ab}
Flavour		8.7 ± 0.000^a	8.4 ± 0.058^b	8.0 ± 0.058^c	8.6 ± 0.000^a	8.6 ± 0.058^a
OAA		8.5 ± 0.058^a	8.2 ± 0.058^{cd}	8.1 ± 0.058^d	8.4 ± 0.058^{ab}	8.3 ± 0.058^{bc}
Colour	30	-	-	7.9 ± 0.058^c	7.9 ± 0.058^c	8.3 ± 0.000^b
Appearance		-	-	7.3 ± 0.000^c	7.3 ± 0.058^c	8.0 ± 0.000^b
Consistency		-	-	7.5 ± 0.000^b	7.5 ± 0.058^b	8.0 ± 0.058^a
Flavour		-	-	7.3 ± 0.058^b	7.3 ± 0.000^b	8.1 ± 0.000^a
OAA		-	-	7.5 ± 0.100^b	7.5 ± 0.058^b	8.1 ± 0.058^a
Colour	60	-	-	-	-	8.0 ± 0.058^b
Appearance		-	-	-	-	7.6 ± 0.058^b
Consistency		-	-	-	-	7.9 ± 0.058^b
Flavour		-	-	-	-	7.9 ± 0.000^a
OAA		-	-	-	-	7.9 ± 0.058^a
Colour	90	-	-	-	-	7.9 ± 0.000^a
Appearance		-	-	-	-	7.5 ± 0.100^b
Consistency		-	-	-	-	7.2 ± 0.100^b
Flavour		-	-	-	-	7.3 ± 0.058^b
OAA		-	-	-	-	7.5 ± 0.058^b

Means with the SD superscripts in a column vary significantly ($p < 0.05$)

Conclusions

The study was proposed to study the process induced effect on the formation, accumulation and retention of volatile and non-volatile flavour compounds in mango nectar. The RTD mango nectar was subjected to thermal, PEF and combination treatment. It was observed that the thermal treatment, as such or in combination, has its influence on both: reduction in retention of volatile flavour compounds and increase in HMF concentration in the processed sample, which was dependent on the time of exposure. The concentration of HMF was also noted to be higher in the thermally treated sample when compared to that of the PEF and combination processed sample. The volatile composition analysis and the HMF estimation (before and after storage) of the PEF processed sample, indicates that the process induced change in the quality of the product is minimal when compared to thermal treatment. It was also observed that the volatile composition of the PEF treated sample was insignificantly ($p > 0.05$) differing from the control sample, indicating the fresh-like character of the product. The study in accordance with the other authors proves the efficiency of the PEF process to produce high quality fresh-like product with less induced changes/ alteration.

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