

Impact of Nonthermal Pulsed Electric Field on Bioactive Compounds and Browning Activity in *Emblica officinalis* Juice

Vasudha Bansal, M. L. Singla, and C. Ghanshyam

Abstract—The effect of nonthermal pulsed electric field (PEF) and thermal treatment (90°C for 60s) was studied on quality parameters of *Emblica officinalis* juice for the period of 6 weeks at 4°C using monopolar rectangular pulse of 1μs width. The PEF treatment was given using static chamber at 24kV/cm for 500μs. The quality of *Emblica officinalis* juice was investigated in terms of non enzymatic browning index (NEBI), 5-hydroxymethyl-2-furfural (HMF), total polyphenol content and antioxidant capacity. °Brix, pH and conductivity were evaluated as physical parameters. The aim of the work was to investigate the effect of PEF on the retention of bioactive compounds and retardation of browning activity. The results showed that conventional thermal treatment had led to a significant ($p < 0.05$) decrease of 48.15% in polyphenol content (129.56 mg of GAE L⁻¹), with higher NEBI and HMF formation ($p < 0.05$) whilst PEF suppressed NEBI and retained higher polyphenol compounds (168.59mg GAE L⁻¹) with limiting the loss to 32.56% along maximum free radical scavenging activity (92.07%). However, pH, °brix and electrical conductivity of treated juice samples remain unaffected. Therefore, PEF can be considered as an effective nonthermal treatment for retaining bioactive compounds along suppressing browning of emblica juice.

Keywords—*Emblica officinalis* juice, Free radical scavenging activity, Pulsed electric field, Total polyphenol content.

I. INTRODUCTION

TRADITIONALLY, juices have been given thermal treatment. The large amount of energy gets transferred during thermal treatments that provide the microbial safety at the cost of nutrients and ruin the organoleptic properties of the food products [1], [2]. However, in current scenario, consumers are not only looking for taste but also demanding food products with high nutritive and functional value. To convince these demands global manufactures are looking for processes suitable of retaining nutrient content along with functional quality of treated foods. Interest in herbs and their extract is on the rise due to their ample therapeutic benefits as lifestyle disorders in the form of diabetes mellitus, hypertension, cardiovascular diseases, obesity, Alzheimer's, psychiatric disorders and cancers are burgeoning in large numbers [3], [4]. Retained potential functional properties of herbal extracts would add to their usage in food additives.

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Emblica officinalis is also known as an Indian Gooseberry and is the richest source of vitamin C and acts as free radical scavenger [5]. This herb has potent pharmacological properties in terms of anti-inflammatory, anti-oxidative, chemoprotective, hypoglycemic and anti-hyperlipidemic [6]. Its extract has also been used in several nutraceuticals in order to enhance their nutritive and functional value. There has been a wide usage of emblica fruits and are consumed as raw, pickled, and their extracted juice have many ayurvedic preparations.

Nonthermal technologies such as high pressure processing, pulsed electric fields, ultra violet radiations, ionizing radiations and pulsed light may fulfill freshness and nutritive food requirements along with food safety. PEF renders low temperature to food products and is emerging as a potential nonthermal technique which can provide the industry a feasible alternative for processing of beverages and seems to be economically efficient as well. Studies have been reported on the role of PEF in preservation of fruit and vegetable juices and observed the promising results in terms of their shelf life while keeping their nutrients intact [7], [8].

However, herbs are considered both for their functional and nutritive value and effects of PEF on the herb juice could amplify its further usage. Since use of higher temperature in thermal treatment degrade the functional compounds where as PEF can restore them owing to their treatment at low temperature. In addition, spontaneous thermal decomposition of ascorbic acid is the major cause for ascorbic acid browning. Therefore, aim of the research is to evaluate the effect of PEF processing on the functionality quality of emblica juice along retardation of enzymatic and non enzymatic browning in comparison with thermally treated samples while taking the fresh sample as control.

II. MATERIAL AND METHOD

A. Chemical Reagents

2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, 5-hydroxymethyl-2-furfural, thiobarbituric acid and trichloroacetic acid were purchased from Sigma Aldrich (St. Louis, USA). Folin Ciocalteu reagent, anhydrous sodium carbonate, methanol, and ethanol were purchased from Merck (Darmstadt, Germany).

B. Emblica Juice Sample Preparation

Fresh fruits of *Emblica officinalis* were purchased from a local supermarket (Chandigarh, India) and were washed, shredded and crushed, the collected juice was then filtered using 2mm mesh steel sieve, and stored at -20°C during pre and post processing. The post processing juice preparation was initiated with thawing the juice at room temperature and centrifuged at 10,000g for 15min and filtered using 0.45µm Millipore filter. Then the juice was filled in polypropylene bottles and stored at 4°C for 6 weeks. The PEF and thermally treated samples were also kept at the same storage conditions.

C. Pulsed Electric Field (PEF) Treatment

PEF treatment was carried out in a laboratory scale static pulsed generator (TLG (S)-01/MN-02, Samtech Ltd, Glasgow G1 1XW, UK). The treatment system provides a monopolar rectangular-wave pulse of 1µs pulse width and was consisted of a stainless steel electrodes separated by a distance of 10 mm. Actual voltage applied was measured with a high-voltage probe (Tektronix TDS1001B). Samples were placed in 5ml chamber. Before placing the samples, the chamber was autoclaved (15 lbs at 121°C). PEF treatment was given at 24 kV/cm for 500µs (Table I). The maximum temperature reached during PEF processing was 36°C which was measured using infrared and contact thermometer (Fluke, 568). Between treating the different samples, treatment chamber was cleaned thrice with distilled water. Immediately after the treatment, samples were filled in 15 ml polypropylene bottles and kept at 4°C for carrying out the analysis.

D. Thermal Treatment

In order to get conventional pasteurized juice, fresh 20ml samples of emblica juice were heated in glass tubes in a thermostatic water bath operating at 110°C. It took 50s to reach 90±2°C and it remained at this temperature for 60s. Then treated samples were immediately cooled to 20°C using ice water bath. After cooling, the samples were poured in 15 ml polypropylene bottles and were stored at 4°C for 6 weeks.

TABLE I
TREATMENT PARAMETERS OF PULSED ELECTRIC FIELD (PEF) USING PILOT
SCALE STATIC CHAMBER

PEF Processing Parameters	
Pulse Profile	Monopolar, rectangular
Pulse Width	1µs
Electric Field Strength	24 kV/cm
Treatment Time	500µs
Pulse Repetition Frequency (PRF) range	1Hz
Maximum Energy per pulse	18J
Generator output Impedance	12.5Ω

E. Total Polyphenolic Content Determination

Total phenolics were determined using Folin-Ciocalteu reagent [9]. Samples were centrifuged at 2000g for 5min at 4°C and diluted by a factor of 50 with distilled water. 100µl of the diluted sample was mixed with 0.75ml of Folin-Ciocalteu reagent (previously diluted 10 fold with distilled water) and

allowed to stand at room temperature for 5min. Then 0.75ml of sodium bicarbonate (60g L⁻¹) solution was added. The solution was incubated for 2 hours at room temperature and absorbance was measured at 765nm using HITACHI (U-3900H) Spectrophotometer. Results were expressed as mg of gallic acid equivalents (GAE) L⁻¹ of the juice.

F. Antioxidant Capacity

Antioxidant capacity was measured using DPPH (2,2-diphenyl-1-picrylhydrazyl) assay for evaluating the free radical scavenging activity of the samples [10]. The emblica juice was centrifuged at 6000g for 15min at 4°C and 0.01ml of the supernatant was added to 3.9ml of methanolic DPPH solution (0.025g L⁻¹) and 0.090ml of distilled water. The mixture was shaken vigorously and kept in darkness for 30min. Absorbance of the samples was measured at 515nm against keeping the blank of methanol. DPPH initial absorption value was taken as control. Antioxidant capacity was calculated as percentage of the radical DPPH inhibition with respect to decrease in absorption of control value.

G. Non Enzymatic Browning Index (NEBI) Determination

Reference [11] was followed to determine the NEBI of the juice during pre and post processing. 10ml of emblica juice was mixed with 10ml of ethyl alcohol (950g L⁻¹) and centrifuged at 2000g for 15min at 18°C. The supernatant was filtered using 0.45µm Millipore filter and the absorbance was measured at 420nm.

H. 5-Hydroxymethyl-2-Furfural (HMF) Determination

It was determined by the method described by Cohen, Birk, Manheim, and Saguy [12]. 5ml of ethyl alcohol (950g L⁻¹) was mixed with 5ml of emblica juice and centrifuged at 8000g for 10min. Then, 2ml of the supernatant, 2ml of trichloroacetic acid (120g L⁻¹) and 2ml of thiobarbituric acid (3.60g L⁻¹) and were mixed in a 16ml screw-cap test tube. The tubes were placed in hot water bath at 40±0.5°C for 50min and then cooled immediately to around 25°C. Absorbance of the samples was measured at 443nm. A calibration curve of HMF was used to quantify the concentration of HMF.

I. Statistical Analysis

Statistical analysis was carried out using analysis of variance (ANOVA). The Tukey test was performed to compare the data and p<0.05 was used to determine statistical significance of experimental study. Co-relation between the parameters was found using Pearson coefficient. The experiments were independently repeated in triplicate sets. All calculations were performed using SPSS® version 15 (Statistical Packages, Chicago, IL).

III. RESULTS AND DISCUSSION

A. Effects of PEF Processing on Total Polyphenolic Content

Polyphenolic compounds are considerably present in fruit and vegetable juices as well as richly endowed in herb extracts. They are known to play an eminent role in scavenging

free radicals along contributing to organoleptic properties [13]. Concentration of total phenolic content in fresh emblica juice was found to be 250mg GAE L⁻¹ of the juice. Reference [14] reported similar results in the extracts of Indian gooseberry. Pronounced decrease in phenolics concentration of about 48.17% ($p < 0.05$) was observed in thermal treatment as compared to 32.56% in PEF treated juices at 24kV/cm for 500 μ s. Reference [8] was in agreement with the concentration of polyphenolic where applying thermal treatment found a decrease of 32.2% of phenols in apple juice in relation to 14.49% reduction was shown by PEF at 35kV/cm. The loss of phenolic content impairs the quality and affects the color and flavor of the juices [15].

The higher retention on application of PEF owing to lower treatment temperature in relation to 90°C applied for thermal treatment as bioactive compounds are vulnerable to high temperatures. Emblica juice retained the concentration of 168.59mg GAE L⁻¹ at 24kV/cm as compare to 129.56mg GAE L⁻¹ in the thermal treatment during the storage of 6 weeks (Fig. 2).

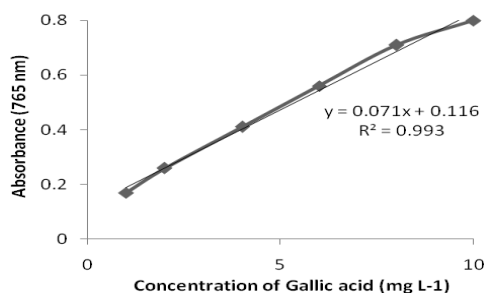


Fig. 1 Standard Curve for Determination of Total Polyphenolic Content

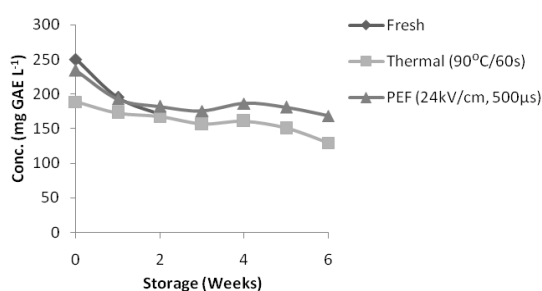


Fig. 2 Effects of PEF processing on Total Polyphenolic Content of *Emblica officinalis* Juice

PEF applied at higher intensity may liable to inactivate the oxidative enzymes as phenol-oxidase, oxidase responsible for oxidation of bioactive compounds and at the same time provides nonthermal treatment that prevents the degradation of heat labile compounds as phenolics and organic acids. Phenolic compounds which are broadly distributed in plant kingdom influence the taste of the juice and are also called as secondary metabolites. These compounds act as substrates for

phenol oxidases that hydrolyze these compounds from mono phenols to o-diphenols and then to o-quinones [16]. The values mentioned in Fig. 2 signifying the effect of PEF on the concentration of phenolic content of emblica juice.

B. Effects of PEF Processing on Antioxidant Capacity of Emblica Juice

Free radical scavenging activity of emblica juice is attributed due to the presence of polyphenolic compounds [17]. Free radical inhibition of untreated emblica juice was observed to be 93.46% and result was in agreement with the range of emblica extracts reported in literature [18] where as reduction of 91.65% was found in thermally treated sample on the same day of treatment.

TABLE II
CHARACTERIZATION OF EMBLICA JUICE: UNTREATED, THERMAL, AND PEF TREATED SAMPLES DURING THE STORAGE OF 6 WEEKS AT 4°C

Treatment	HMF (mgL ⁻¹)	%DPPH
Untreated	0.50 ± 0.3	93.46 ± 0.006
Thermal (90 °C/60 s)	7.95 ± 0.6**	89.42 ± 0.006*
PEF (24kV/cm) for 500 μ s	2.49 ± 0.1**	92.07 ± 0.002**

93.12% of free radicals were found to be inhibited on the immediate day of PEF treatment at 24kV/cm. After the storage of 6 weeks, inhibition of free radicals was decreased to 89.29% ($p < 0.05$) in thermally treated sample (Table II) while PEF retained significant higher antioxidant activity as 92.07% ($p < 0.01$) at 24kV/cm. Similar pattern of significant reduction of antioxidants during thermal treatment was reported in orange juice and gazpacho-vegetable soup [10] and also observed higher DPPH inhibition (40.6%-47.4%) with application of 50 Hz monopolar pulses of 2 μ s width at 15kV/cm and 25kV/cm for 100-400 μ s. Reference [19] showed that antioxidant activity was more related to total phenols in grapefruit juice. Reference [20] studied the effects of pulsed electric fields at varied intensities at different treatment time on bioactive compounds of watermelon juice and reported antioxidant retention of 80.9% to 85.5% at 25kV/cm for 50 μ s with monopolar pulse of 1 μ s width as compare to 80.0% to 82.2% of antioxidant retention at 25kV/cm for 2050 μ s with same monopolar pulse of 1 μ s pulsed width. Thus, retention found to be increased with monopolar pulse with increased treatment time which keeps the free radical scavenging activity of biocompounds in the range with respect to fresh juice.

C. Effects of PEF Processing on Non Enzymatic Browning Index

NEBI is an indicator of quality which reflects the sensory properties and higher absorbance values indicate browning over period of storage. Browning values were accelerated on the application of thermal treatment (90°C for 60s) as compared to PEF treated juice. PEF treated juice at 24kV/cm for 500 μ s achieved the lowest absorbance values ($p < 0.05$) and stabilizes the juice till the end of 6 weeks (Fig. 3). Thermally treated juice was found with consistent increase in browning throughout the storage period, as on heating, phenolic

compounds form complexes with proteins, which increase the turbidity of thermally treated emblica juice. Reference [21] showed a significant ($p < 0.05$) prevention of apple and cranberry juice blend degradation with the application of nonthermal processing where as thermal treatment caused a significant darkening of the same product. Thus, these browning reactions lead to unappetizing tastes and palatability of vitamin C is lost [22].

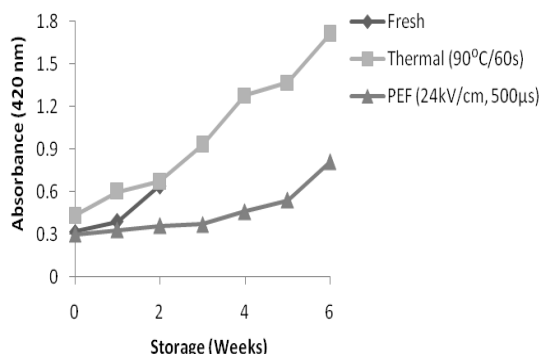


Fig. 3 Effects of PEF on Non Enzymatic Browning Index of *Emblica officinalis* Juice

D. Effects of PEF Processing on Formation of HMF

Heat is the key player in formation of HMF in juices of fruits and vegetables. PEF has proved the most appropriate nonthermal candidate for keeping it below the standard values and it is used to compare the deterioration of juices with respect to thermal treatment. Maximum values of 5 mg L^{-1} are allowed in juices of fruits and vegetables [23]. Vitamin C browning leads to degradative changes and results in formation of furfurals [24]. Formation of 5-hydroxymethyl-2-furfural (HMF) reduced significantly ($p < 0.05$) at 24 kV/cm PEF treated emblica juice (Table II). However, thermal treatment (90°C for 60s) showed a significant rise ($p < 0.01$) in HMF formation ($7.95 \pm 0.6 \text{ mg L}^{-1}$) during the same storage period. Formation of HMF has been the major criteria in evaluation of color and flavor. Reference [25] exhibited similar results.

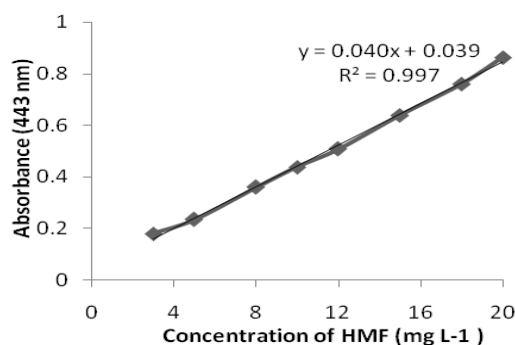


Fig. 4 Standard Curve for Determination of 5-hydroxymethyl-2-furfural Content

E. Effects of PEF Processing on Physical Parameters

The effects of thermal and PEF treatment on emblica juice based on physical parameters such as pH, °brix and conductivity are shown in Table III. Juices are acidic in nature and their low pH prevents the spoilage caused by micro-organisms. pH of all the treated samples were lie in the range (2.75-2.79). However, no significant differences ($p > 0.05$) were observed during PEF and thermally treatment with respect to untreated juice. °Brix signifies percent of soluble solids (TSS) in the juices and responsible for enhancing the palatability. Neither PEF nor thermally treated juice showed significant differences ($p > 0.05$). Conductivity of the food products represents the medium for passage of electric conduction. The differences between the mean values of treated samples were insignificant ($p > 0.05$). Reference [26] was in concordance with the observation.

TABLE III
CHARACTERIZATION OF PHYSICAL PARAMETERS

Treatment	pH	°Brix	Conductivity (Sm^{-1})
Untreated	2.72 ± 0.09	11.72 ± 0.90	0.371 ± 0.85
Thermal (90 °C/60 s)	2.79 ± 0.05	11.83 ± 0.20	0.378 ± 0.44
PEF (24kV/cm) for 500µs	2.76 ± 0.02	11.70 ± 0.25	0.369 ± 0.86

Values are means \pm standard deviations (SD) of triplicate repeated sets of juice. Letters in superscript correspond to level of significance of differences. ** $p < 0.01$; * $p < 0.05$. [HMF: 5-(hydroxymethyl)-2-furfural; DPPH: (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity].

IV. SUMMARY

We found that it is possible to inactivate enzymatic reactions at electric field strength of 24 kV/cm for $500 \mu\text{s}$ for sustaining potential benefits of emblica juice. Nevertheless, PEF is a persuasive treatment for conserving its physicochemical characteristics. Therefore, PEF parameters could be used as promising treatment for retaining bioactive compounds along their functional quality of emblica juice.

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