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Effect of PEF on the Characteristic Quality of Fruit and Vegetable Juices as Compared to Thermal Processing: A Review

Aim: Application of PEF Technique in Fruits and Vegetables Juices and its Effect on Shelf-life and Keeping Quality

Divya A C[1], Mahenoor J Mulla[2], Keerthana S[3]
Department of Food Technology
MSRUAS
Bengaluru, India

Abstract:- PEF (Pulsed Electric Field) is a novel emerging technology which is believed to have high potential in extending the shelf-life of food products especially liquid foods. PEF technique have been studied in juice industry. The working principle of PEF is based on the application of pulses of high voltage to liquid foods placed between two electrodes. Several studies show that PEF processing delivers safe and chill stable fruit and vegetable juices with fresh-like sensory and nutritional properties. PEF is a productive technique which improves extractability and recovery of nutritionally valuable compounds also the bio-availability of micro-nutrients in a diverse variety of liquid foods.

The aim of the present review article is to summarize the application of PEF on different fruit and vegetable juices in extending the shelf-life, microbial and physico-chemical quality.

Keywords:- *PEF, Juice, Quality, Application, Food industry, microbial load, storage and Shelf-life.*

I. INTRODUCTION

In this review we would like to compare the quality of different juices and their quality. Selecting five research papers on the effect of quality of juices (Grapefruit juice, orange juice and spinach juice).

Recently, food industry is developing innovative non-thermal techniques, such as Pulsed Electric Fields (PEF), High Pressure Processing (HPP) and Ultrasound (US) as a green alternative for food processing (Picart-Palmade et al., 2018). Research shows Non-thermal techniques are more energy cost-effective, environment-friendly, and efficient for product quality and microbial safety (Manzoor et al., 2019). They also improve the quality of products and eliminate or reduce harmful impact on the environment. Non-thermal techniques also ensure a significant decrease in microbial activity, higher shelf life and safe consumption of food products (Ahmad et al., 2019; Zhang et al., 2019).

PEF treatment involves the application of short pulses (µs to ms) of electric fields in the order of 100–300 V/cm to 20–80 kV/cm, which induces permeability variation of biological membranes due to pore formation. (Dourado et al., 2019).

PEF is widely used for liquid foods such as juices and has positive results for enzyme inactivation and microbial reduction (Khandpur & Gogate, 2016). Some studies have also shown that PEF is useful for retaining bioactive compounds of liquid foods (Leong, Burritt, & Oey, 2016). PEF treatment has also shown to be very effective for inactivation of microorganisms, increasing the pressing efficiency and enhancing the juice extraction from fruits and vegetables and for intensification of the food dehydration and drying (Gulyi et aI., 1994; Barbosa-Cánovas et aI., 1998; Barsotti and Cheftel, 1998, 1999; Estiaghi and Knorr, 1999; Vorobiev et aI., 2000, 2004; Bajgai and Hashinaga, 2001; Bazhal et aI., 2001; Taiwo et aI., 2002).

In this review, we have focused on the effect and application of PEF treated citrus juice in retaining the bioactive compounds and reduce microbial activity and increase of keeping quality; and effect of PEF treated vegetable juice on enzymes, reduction in aflatoxins and comparing them with thermal processes.

II. WORKING PRINCIPLE OF PEF

PEF is a novel technology which includes the application of microsecond (µs) pulses of high electric field to a material placed between two electrodes (Fortuny R S et. al., 2009). The PEF system for the treatment of pumpable fluids is composed of a PEF generation unit that consists of a high voltage generator, a pulse generator, a treatment chamber, a product process system and a set of monitoring and controlling equipment (Barbara F J et. al., 2015) (Fig. 1). With the help of energy transfer to the fluid PEF treatments causes an increase in temperature and electrical conductivity of the treatment medium. The temperature increase affects the viscosity and stability of the cell membrane and changed conductivity at constant energy input diminishes the field strength (Q X Ou et. al., 2017).

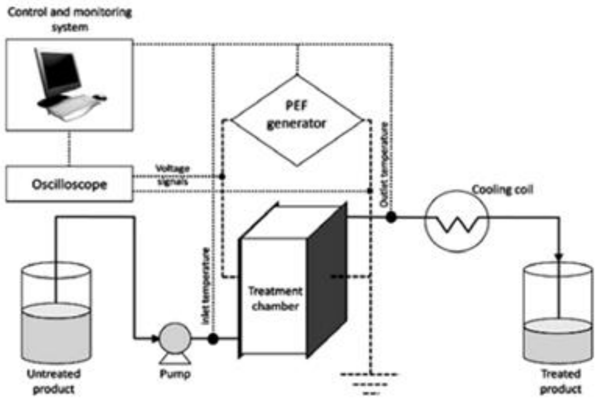

Fig 1:- PEF Processing system for pumpable products like juice.

III. METHODOLOGY AND RESULTS

A. Effect of PEF on Grapefruit juice:

The main aim of this study was to see the effect of PEF+US treatments on grapefruit juice for it's quality and shelf-life.

Rana Muhammad Aadil et. Al., (2017) studied the combined effect of the Pulsed Electric Field and ultrasound on bioactive compounds and microbial quality of grapefruit juice. Here, Grapefruit was extracted after washing it thoroughly in a juice extractor. Then it was treated with PEF at the flow rate of 80 ml/min, pulse frequency of 1 kHz, 20 kV/cm electric field strength at temperature 40^0C for 600 µs (Continuous method as mentioned by Aadil et al., 2015) followed by Ultrasound treatment in an ultrasonic bath cleaner radiating 600 W at frequency of 28 KHz and 20^0C for 30 min (cited prior by Aadil et al. 2015). In bioactive compounds Total carotenoids (Liao et al.2007), lycopene (Oliu, Serrano, Fortuny, and Belloso 2009), total anthocyanin content (pH-differential method described by Lee, Durst, and Wrolstad, 2005), total phenols, flavonols and flavanoids and anti-oxidant capacity was determined for the PEF treated juice using the methods well described by standards. The microbial load was tested refering to FDA's Bacteriological Analytical Manual (FDA, 2001) The methods used were Total plate count (TPC) using nutrient agar medium counted by pour plate method, and yeast, and mold (Y&M) counts were performed on Potato Dextrose Agar medium. The results were then depicted as log colony-forming units (CFU/ml) of grapefruit juice which is discussed in the further section. The juice was then compared with the control sample, Ultrasound treated sample, PEF treated sample and US+PEF treated sample.

Barbosa-Canovas, Pothakamury, Gongora-Nieto, and Swanson (1999) who revealed that PEF treatment reduced the activity of Y&M due to electroporation and electrofusion, this was used as a reference to see the effect of PEF+US treatment on bioactive compounds of grapefruit. The result showed that there was a significant decrease in the microbial activity, in all the treated samples (PEF, US & PEF+US). But the highest inactivation was noted in the case of PEF+US that shows the higher reduction in efficiency of microbial cells in grapefruit juice. Similarly, the total lycopene content and anthocyanin content were reported to be increased in this study, which held true when the authors compared it to other similar research studies done by Odriozola-Serrano et al., 2008 using strawberry juice and Knorr, 2003 using grape juice. The authors agreed to the conclusion that the significant increase of lycopene and anthocyanin content might be due to the cavitation process that regulates various chemical or biological reactions including increase in the diffusion rates and disintegration of affected particles by Tiwari et al., 2009. Also, the total carotenoid (TC) content was also seen to be increased in PEF+US treated sample as compared to other samples. The authors concurred that the increase in the TC might be due to the ruptured cell walls during cavitation process resulting in release of free carotenoids as seen by Abid et al., 2014. The authors also compared the result to other similar research studies done by Jabbar, Abid, Hu, Muhammad Hashim, et al., 2014 using carrot juice (US+HPP). The total antioxidant capacity (TAC) and DPPH free radical activity was increased in US+PEF treated sample, this maybe due to the improved level of phenolic compounds that produce cavitations after US treatment as noticed by Aadil et. Al., 2013. The highest level of TAC and DPPH was observed in PEF+US which the authors concurred might due to the synergistic effect of both treatments in grapefruit juice. Total flavonoids, flavonols and phenols as shown in the figure below:

Samples	Total flavonoids (Catechin equivalent µg/g)	Total flavonols (Quercetin equivalent µg/g)	Total phenolics (Gallic acid equivalent µg/g)
Control	640.10 ± 0.05c	2.19 ± 0.02c	820.00 ± 0.05c
US	700.37 ± 0.04b	2.95 ± 0.03b	909.51 ± 0.07b
PEF	701.10 ± 0.05b	2.94 ± 0.04b	909.53 ± 0.04b
PEF+US[a]	730.19 ± 0.05a	3.30 ± 0.04a	946.54 ± 0.05a

Fig 2:- Showing results of TF, flavonols and phenol content (Aadil R M et. al., 2017).

The highest levels of TP, TF, and total flavonols in PEF+US treatment may be due to the complementary effects of both techniques on grapefruit juice. The author concurred that the increase in total phenols maybe due to the Ultrasound treatment which releases the phenolics from ruptured cell membranes mainly owing to the cavitation process by Aadil et al., 2013. When the authors compared the results with the similar research studies, elevation in TP contents was observed in sonicated kasturi lime juice by Bhat, Kamaruddin, et al., 2011 and PEF treated Tempranillo grape juice by Lopez, Puertolas, Condon, Alvarez, & Raso, 2008.

So, to conclude, this research depicts the effect of the PEF+US treatment on the quality (bioactive compounds) and microbial safety of grapefruit juice. PEF+US treatment resulted in improvement of DPPH activity, TAC, flavonols, TF, TP, and reduction in microbial load and the combined effect of Pulsed Electric field and Ultrasound treatments showed the highest reduction in microbial activity than a single treatment of US and PEF.

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B. Sterilization of PEF for Green Juice to Retain Nutrient:

The aim of this study is to apply PEF a non-thermal technology for sterilization of green juices, without impacting the quality.

Koki Saito and Yasuhito Nozawa in their research article titled- "Development of Non-thermal Sterilization Treatment System without Impacting to Enzyme in Vegetable Drink by Pulsed Electric Field" in Electronics and communication in Japan , vol.101 , No. 4, 2018 signifies the development of non –thermal sterilization treatment system without impacting the enzymes in vegetable drink by PEF. Saito K et. al. developed the design of small scale circulating sterilization system by measuring green juice sterilization and Barley grass juice sterilization rate by decay waves and parallel plate electrodes. Coaxial cylindrical electrodes were used for circulating sterilization. The reason is that the coaxial cylindrical electrodes is that the sample solution necessarily flows between the electrodes during circulation, so that no sample untreated by electric filed remains, as distinct from the parallel plate electrodes. Heat is released between the electrodes due to high repetition rate of electric field pulses, providing water flowing between the electrodes at 10°C. PEF was applied at repetition frequency of 24 pps and pulse width of 4.0 µs, with 2 L of green juice circulating at flow rate of 6.5 L/min. 1379 pulses of electric field was applied to the entire juice, 80 shots of PEF was required at field strength of 30 kV/cm, which corresponds to 110,320 pulses as given by Saito K et. al. in case of circulating sterilization. Further, electric field for 120 min at 30 kV/cm and for 60 min at 40 kV/cm was carried out in order to examine sterilization.

Result obtained from the **fig.3.** for green juice shows the survival ratio can be reduced to about 13% at field strength of 30 kV/ cm. Therefore, no further decrease in survival ratio was observed after 60 min. Combining the PEF and control bacterial culture, test was performed, by applying t-test at significant level of 5% to obtain the results with treatment time of 40 min and 60 min so as to check whether sterilization advances over time. Therefore no significant difference was observed in t-test even after 60 min, fact that survival ratio remains almost unchanged after 60 min. Combining results, it was concluded that electric field of 40kv/cm, survival ratio can be reduced to about 2.3%. Thus for small-scale bacteria it's difficult to sterilize at 30 kV/cm were successfully sterilized when electric field strength was increased, and the survival ration dropped below 10%. It was confirmed that enzymes included in the green juice was not deactivated when Intense Pulsed Electric Field (IPEF) was applied.

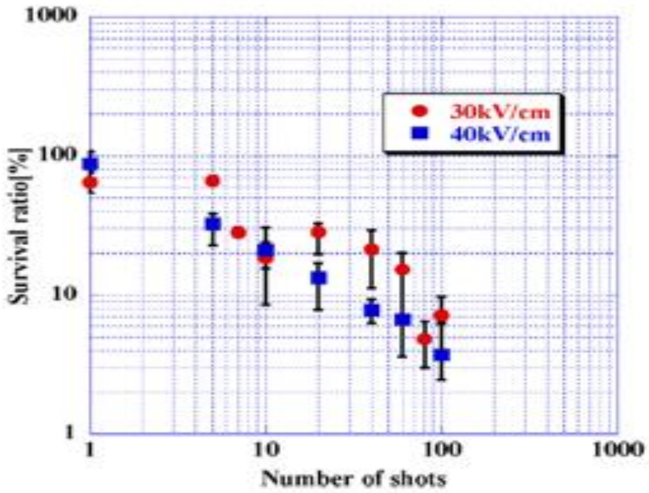

Fig 3:- Shows the sterilization effect for Decay wave form (Saito K et. al., 2018).

This study further shows the effect of thermal sterilization and the effects on enzymes contained in green juice was examined by combining with PEF sterilization. Performing survival ratio after heating green juice for 1 min at 40 °C, 50°C, 55°C ,60°C using constant temperature bath in order to determine thermal sterilization temperature providing same sterilization rate in case of electric field sterilization to examine how green leaves of barley juice effects these temperatures. To examine the change in enzymes, PEF application in the sterilization evaluation system as well as t-test was performed to check the Quantity changes of Super-oxide disbutase(SOD) for decay waves and for electric filed strength at 30 kV/cm and 40 kV/cm at pulse electric field sterilization.

Results obtained from the effect of thermal Sterilization – from **fig.4** for Barley grass juice can be illustrated that condition of thermal sterilization at 40°C- 60°C is required. The chopped barley grass settled out thus making two-layer structure, and the color changed, this can be attributed to coagulation of chlorophyll contained in the young grass. In contrast in case of circulating sterilization at pulse electric field strength of 40 kV/ cm for 60 min, neither deposition nor pigment change was observed. Effect on enzymes contained in green juice was be analyzed from the **table 1.** It can be observed that the content was 4158U/mL and 3226 U/mL before and after field application, while t-test showed no significant difference, this indicated that an enzyme is not affected by decay wave forms. Results obtained for the change in enzymes content after PEF application in the small scale circulating sterilization system as well as by combining t-test from the **table 2.** Therefore, the content before and after electric field application was respectively, 3584U/mL and 4229 U/mL at 30kV/cm , and 2457 U/mL and 3184 U/mL at 40 kV/cm. These findings demonstrated that circulating sterilization with decay wave forms rectified the problems of conventional sterilization, implying that there is no affect on quality.

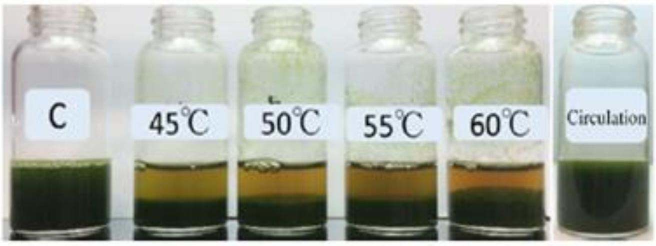

Fig 4:- States of green juice at each temperature of heat sterilization and circulating treatment by pulsed electric field sterilization (Saito K et. al., 2018).

	Before applying	After applying	*t*-Test
Decay wave	4158	3226	No differential

Table 1:- Quantity changes of SOD for decay wave at pulsed electric field sterilization (Saito K et. al., 2018).

	Before applying	After applying	*t*-Test
30 kV/cm	3584	4229	No differential
40 kV/cm	2457	3184	No differential

Table 2:- Quantity changes of SOD for electric field strength at pulsed electric field sterilization (Saito K et. al., 2018).

PEF sterilization aiming at non-thermal sterilization technology does not influence the nutrients and enzymes in the green juice production. From the above results, we can confirm quality by examining the changes in pigment and enzymes content as a result of PEF sterilization combining with thermal sterilization.

C. Development of novel processing techniques for the quality and safety improvements of spinach juice:

The main aim of this study is to investigate the relevant changes that occurs during US and PEF treatment, as well as acceptable alternative methods for spinach juice.

Muhammad Faisal Manzoor and Zahoor Ahmed in this study, combined the effect of Ultrasound (US) and pulsed electric field (PEF) techniques analyzed the quality improvement and microbial safety of spinach juice. The spinach juice was treated with US at frequency of 40 kHz, radiating power of 200 W below 30 ± 2 °C temperatures for 21 min in ultrasonic bath cleaner, and PEF treatment of pulse frequency of 1 kHz. Flow rate 60mL/min, temperature at 30± 2° C for 335 µs with an electric field strength of 9kV/cm. For the combined treatment (US+PEF), the sample was treated with US and then with PEF. The resultant mixtures after all treatments were passed through a sterilized double layer muslin cloth. Final spinach juice was stored at 4°C for further analysis. Same effects were applied to check the nutrition composition of spinach juice. The free amino analysis of spinach juice samples was performed using A300 amino acid analyzer. A sample of 4ml was mixed with 1 ml of dinitrosalicylic acid and incubated at 4° C for 60min. The mixture was centrifuge twice at 9,000 rpm for 15 min, filtered. The supernatant was diluted with a diluents solution to final amino nitrogen from 0.008 % to 0.01% concentration. Free amino acids were separated on a liquid chromatographic column and detected through ninhydrin reaction. To check the mineral elements for nutrition composition of spinach atomic absorption spectroscopy was used (Hitachi Z, 2000). A digestion mixture (7:1) of 65% HNO_3 and 30% of H_2O_2 was used to digest 1 mL of juice sample in Teflon vessel using cathode lamp radiation in microwave. The digest mixture was diluted with distilled water and to make volume of 50 ml for each mineral the standards were made within the concentration range of mineral elements. To examine the microbial load, 3M petriplates tests were used. The microbial loads of aerobic count plates, *coliform*/*E-coli* and yeast and mold of spinach juice was assessed.

Results obtained were studied as follows for Amino Acids, Mineral content and Microbial Load; Free amino acids produced on processing treatments that make the product biologically active. The concentration of free amino acids (FAA) in untreated, US, PEF and US+PEF treated spinach juice was examined and the result indicated that there was a significant ($P< 0.05$) increase in the total FAA of the US and US+PEF treated spinach juice as compared to untreated juice, while significant ($P<0.05$) decrease in FAA of PEF treated spinach juice. PEF may result in the conjugation of amino acids leading to a reduction of free amino acid concentration. The authors concurred that the higher total FAA contents in the sample subjected to US is due to the release of some FAA from the cell tissues that were ruptured by US which was studied by Jam- brak, Lelas and herceg, 2014. Therefore, the juice sample treated with US+PEF presented the highest value for FAA as compared to untreated, US and PEF treated juice. The increase in FAA contents obtained is due to the synergistic effect of both non-thermal techniques such as PEF and US. For Mineral content, a significant decrease in Fe, Ca, Mn and Zn minerals was observed in US treated sample, while K mineral significantly increased. Similarly, in PEF treated sample, decline was noted in Mn mineral, while a considerable increase was recorded in K mineral. The US+PEF treated juice sample exhibited the highest level of all minerals than in untreated, US and PEF treated juice.

The effect of microbiological analysis, showed a significant reduction in the activity of E.coli/ Coliforms, Y and M (Yeast and Moulds) and TPC (Total Plate Count) during US, PEF And US+PEF. The activity of Y and M , E-coli/coliforms and TPC was significantly reduced on combined US+PEF treatment, PEF and US treatment as compared to untreated sample. The possible reason for the reduction of Y and M and TPC activities is the cavitation process, which cause the combination of physical and chemical breakage. It has been reported that the, cavitation process increases localized heating and produces the production of free radicals which could cause a reduction of microbial loads by Jabbar et al.,2014. Zhang et al., 2019 described that the activity of micro-organisms during PEF treatment reduces due to the electroporation and electrofusion which was then confirmed by M F Manzoor et. al., 2020 in this study. Furthermore, the effect of US+PEF showed the maximum reduction of microbial load than individual US, PEF treatments and untreated samples.

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To conclude, the combined US+PEF application would be preferentially selected over US or PEF alone because of an increase in total free amino acid and mineral content. Overall, the improved nutritional quality achieved through US and PEF treatment makes both technologies suitable for industrial use for spinach juice.

D. Reduction of Aflatoxins in Grape juice by PEF:

The aim of this article is to evaluate the application of PEF and High Pressure Processing(HPP) techniques as useful tool for aflatoxin reduction in grape juice.

Noelia Pallares et. al., 2021 in this study used spiked grape juice samples with aflatoxin to treat it by PEF or HPP method.

Aflatoxin	PEF (30 kV and 500 kJ/kg)		HPP (5 min at 500 MPa)	
	Grape Juice	Water	Grape Juice	Water
AFB1	25 ± 10	30 ± 4	17 ± 2	73 ± 5
AFB2	72 ± 5	75 ± 12	14 ± 3	61 ± 5
AFG1	84 ± 6	84 ± 1	19 ± 6	87 ± 5
AFG2	24 ± 5	31 ± 13	29 ± 10	85 ± 4

Results expressed as % reductions of aflatoxins ± % RSDs (n = 3).

Table 3:- % Reduction of Aflatoxins by Pulsed Electric Field treatment (PEF) vs High Hydrostatic Pressure treatment (HPP) in grape juice and water samples spiked at 100 µg/L (Pallares N et. al., 2021).

A high pressure equipment which is capable to produce nominal pressures upto 680MPa equipped with 2.35L pressure chamber filled with the mixture of water and anti-corrosion additive was employed for HPP treatment. An Elea pulsed electric field cellcrack III equipment was employed for the treatment of PEF. The treatment conditions consisted in voltage of 30kV and average of 238 pulses was applied in various cycles to reach 500KJ/kg.

Noelia Pallares et. al. Came to the conclusion that, HPP treatment can cost disruption of ionic and hydrogen bonding while covalent bonds are not affected. The differences obtained between the two treatments maybe attributed to physico-chemical characteristics, pressure of HPP has minimum effect on breakage of covalent bond and is transmitted instantaneously. The authors concurred that the PEF process shows the disintegration of the cell membrane and the formation of membrane pores, modifying sensorial and nutritional properties of grape juice. It could also influence the structure of amino acid, protein and polysaccharides (Dourado et. al., 2019).

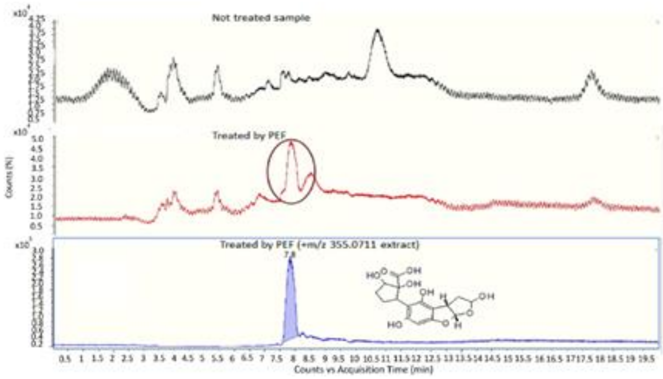

Fig 5:- Chromatogram of degraded aflatoxin *(Pallares N et. al., 2021).*

To conclude, HPP and PEF treatment contributed to significant reduction of aflatoxin in treated grape juices. Both HPP and PEF treatments allowed in a shorter application time similar reduction to those obtained with Thermal processing.

E. Stability of Orange Juice for long term storage by comparing PEF to Thermal processing:

The main aim of this article is to review about processing of orange juice by PEF and Thermal heat treatment carried out to determine the quality variables during storage of orange juice for 180 days @ 4^0C.

E Agcam, A. Akyildiz, G. Akdemir Evrendilek, 2016 in their study used oranges which were washed, peeled, cut into two halves and pressed using a bench-scale automatic orange squeezing machine. In PEF treatment a laboratory-scale PEF OSU-4A system (Evrendilek et. al., 2004) was used for PEF treatment. PEF generator provided with square wave bi-polar pulses and was equipped with six co-field flow chambers. This processing was conducted at 35^0C however processing temperature increased upto 58.2^0C with the increased electric field strength. In heat pasteurization treatment, a bench scale system designed by Agcam et. al., 2014. For preliminary experiments the two heat pasteurization treatments @ 90^0C for both 10 s and 20 s were applied.

The significant changes detected by shelf-life study, the total dry matter and index browning index value of the samples increased towards the end of the storage time. The browning index value increased more for heat treated orange juice than for the PEF treated one. Orange juice samples had lower total dry matter and browning index value when treated with the mild PEF conditions than with the Intense PEF conditions. The PEF and heat processing treatments did not significantly change ascorbic acid content and PEF was found very effective to inactivate pectin methyl esterase (PME) with no PME recovery during storage (Agcam et. al,2014 Yeom et. al., 2000). PEF was also reported successfully to inactivate endogenous microflora, and food borne pathogens (Mc Donald et. al., 2000). Rivas et. al., 2006 and Vervoort et. al., 2011 both detected a very low level of Hydroxy methyl Furfural (HMF) in PEF treated orange juice with storage. In this study it also showed that HMF formation did not occur immediately after the PEF treatment. However, HMF formation remarkably increased for heat treated sample with the storage.

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To conclude, the study showed that mild PEF processing did not cause degradation of the quality attribute, in particular, of ascorbic acid of orange juice. Preservation of ascorbic acid was accomplished using the PEF treatment unlike the heat treatments, the same PEF processing parameters did not cause HMF formation during the entire storage period. Also the sensory properties of orange juice decreased significantly with the storage period such that overall acceptance of the processed orange juice dropped significantly at 180th day at 4^0C. The overall acceptance value was better for the samples treated with mild PEF conditions than those treated with heat (Agcam et. al., 2014).

IV. CONCLUSION

In this review, we have reviewed five research articles that combine the various nutritional parameters and microbial load of various juices such as orange, grapes and spinach. From these, we have concluded that PEF is the most efficient technique used in food processing that extends the keeping quality of the juices. In general PEF cause low temperature increase that prevents heat-induced changes in colour, flavour, taste, and nutrient content of food. PEF gives rise to structural changes of cell membrane and degeneration of membrane permeability barrier by means of electroporation, thus improving the quality and shelf-life of the food product.

V. STUDENT COMMENT

The PEF technology has been advantageous to inactivate micro-organisms, extend shelf-life and increase the quality of juice. It is an innovative technology and eco-friendly that is harmless to the environment. PEF is not widely used in India for commercial purposes and the marketability has to be boosted. Labelling of PEF treated products has to be properly done to increase the knowledge of customers.

VI. FUTURE SCOPE

Although PEF technique has been applied extensively to various food products and their effects studied. The majority of the commercial PEF systems work on the laboratory scale principle with results obtained by it. The generalization of the PEF systems for commercial purposes is yet to be researched upon.

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