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Design and manufacturing of key lime juice processing system by pulsed electric field and evaluation of its quality characteristics

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ABSTRACT

Key lime juice can be a non-fermenting product with high acidity and a rich source of ascorbic acid and phenol compound. In order to maintain the quality of the product, it is important to choose a suitable method for processing this food. The use of modern non-thermal methods to produce high quality products should be considered. In this research, by designing and constructing a continuous pulsed electric field (PEF) system, the effect of this method on the degradation of pectin methyl esterase (PME), ascorbic acid, total phenol compound, cloudiness index, color changes and sensory evaluation in key lime juice is discussed. In this research, a bipolar square wave of 10 microseconds and a constant frequency of 1000 is used. In the processing chamber of this device, two cases of stainless steel with length and thickness of 10 and 0.5 ml, respectively, were used. Key lime juice was processed for 500, 571, 666, 800 and 1000 microseconds with Field intensity of 22.22, 33.33, 44.44 and 55.55 kV per cm. In general, by increasing the field from 22.22 to 55.55 kV / cm at a constant time, the process of reducing the degradation of PME enzyme is normally increased by 25%. Applying the highest electric field of 55.55 in 1000 microseconds caused 14% ascorbic acid degradation compared to fresh samples. With increasing pulsed electric fields, the cloudy index of the sample and browning index have increased. The use of high intensity pulsed electric field increased the degradation of PME enzyme and the cloudy index and reduced lightness compared to the fresh sample. Preservation of sensory properties of key lime juice developed with PEF during shelf life showed that the non-thermal pulsed electric field method can act as a promising approach in products with high acidity and enzyme degradation.

1. Introduction

Lemon fruit is one of the most important citrus fruits from the economic point of view in Iran, which is consumed all over the world today. There are important bioactive compounds in lemon fruit. The most important of these compounds are ascorbic acid, flavonoids, limonoid and various phytosterols. One of the important flavonoid compounds in lemon is rutin, which prevents the activities of free radicals in the body and also has anti-inflammatory, anti-allergic and anti-microbial properties. Lemon juice[1] It is a non-fermented product obtained by mechanical methods from the endocarp of fresh, ripe and healthy lime fruit. The acidity of lemon juice is 5.5 (citric acid) and pH = 2-3.2-8. Lemon juice is used as a flavoring in foods and also in the preparation of various carbonated drinks. In many parts of the world, lemon juice is used in the treatment of many diseases. Lemon juice is a rich source of vitamin C or ascorbic acid, which is estimated at 10.5 mg per 100 grams [1].

Increasing the shelf life of food has traditionally been possible with thermal processing alone or combined with chemical and biochemical preservation methods. However, thermal processing somewhat reduces the quality of the product and its freshness; Therefore, the ideal processing method is a process that deactivates harmful microorganisms and enzymes and stops destructive reactions with minimal damage to the structure and loss of nutrients in the product. Nowadays, the use of new non-thermal processes, including electric pulse fields, to prevent the reduction of fruit juice quality compared to the conventional thermal process, has been considered as a promising approach. The low pH in some fruits, including fresh lemon juice, helps protect it from the growth of pathogenic bacteria. Meanwhile, the use of lemon juice for a longer period of time has led to new public health problems related to the presence of pathogenic microorganisms [2].

Pulsed electric field technology[2]can be considered as a potential alternative to the traditional thermal process of foods. PEF technology is presented as an advantage compared to thermal processes, because it preserves the product by better preserving its color, flavor, texture and nutritional value and eliminates harmful enzymes. The sensitivity of enzymes to the PEF process depends on cell characteristics such as structure and size. In addition, factors such as product pH, water activity (a_{In}), dissolved solids and electrical conductivity affect the amount of enzyme degradation [3].

PEF technology involves the application of high voltage pulses to liquid or semi-solid foods that are placed between two electrodes, and due to which an electric field with high intensity is produced between the two electrodes, a large flux of electric current is created, which is removed from the food without significant changes in the material. Nutrient and sensory properties of food pass through. In the pulsed electric field process, pasteurization of food is done in microseconds. The applications of PEF in food processing are usually considered in two main categories: microbial and enzyme inactivation to increase the shelf life of liquid food and improve mass and tissue transfer in solid and liquid materials. In general, the effects of PEF deactivation in enzymes depend on electroporation of cell membranes and organelles [4]. Applying energy using an electric field and creating a hole in the cell membrane, electroporation[3] they say. The permeability of the cell membrane consists of two stages. Firstly, the applied electric field should induce the formation of pores and secondly, the pores should be stable enough. Typically, food is placed between two electrodes and exposed to an electric field in the form of very short (few microseconds) and high voltage (kilovolt) pulses. One electrode is connected to a high voltage switch and the other to the ground. The electric field strength (E) generated between pairs of electrodes can be estimated by dividing the applied voltage U by the inter-electrode distance d (i.e. $E = U / d$). Electric field strength, treatment temperature, time and input specific energy are the main processing parameters affecting the degree of microbial and enzyme inactivation [5]. In this method, by placing the food in a chamber with two electrodes that

1. Key lime juice
2. Pulsed Electric Field (PEF)
3. Electroporation

create short electrical pulses, it causes the inactivation of the microorganism and enzymes as a result of increasing the storage time of the processed food with minimal effect on their quality. The extent of this effect depends on the intensity of the electric field, process time, waveform and process temperature.

Structural changes in enzymes after the PEF process were observed by Yeom et al. (1999) [6]. But the mechanism of deactivation of enzymes by PEF process is not well understood yet. The two electrochemical and thermal effects associated with PEF individually or synergistically lead to changes in the structure and composition of enzymes and their deactivation [7]. The structure of proteins is stabilized by the balance between covalent and non-covalent peptide bonds, including hydrogen, hydrophobic, electrostatic and van der Waals bonds. The use of external electric fields causes the destruction of peptide chains resulting from electrostatic reactions. In addition, the PEF process stimulates electrolysis and the formation of free radicals. As a result, it causes pH change in liquid environments and oxidation of essential amino acids for the survival of enzymes [8]. Yum et al. (2000) approximately 90% inactivation of pectin methylesterase[4] reported in orange juice by applying a pulsed electric field with an intensity of 35 kV/cm for a treatment duration of 59 microseconds and an output temperature of approximately 60 C ◦ [9].

The stability of the cloudy state plays an important role in the appearance and creating a good mouth feel. According to Stokes' law, particle diameter, particle density, and viscosity are other factors that influence the sedimentation rate and, as a result, the stability of the cloudy state. Turbidity positively affects the color and organoleptic properties of citrus juice. Membranes and compounds with high molecular weight, such as pectin, are suspended in citrus juice during the rupture of endocarp cells during mechanical extraction methods. Pectin is an acid-sugar derived polymer that has a significant effect on the cloudiness stability of fruit juice. The PME enzyme esterifies the methoxylated pectin, which causes the cloudiness of the juice to disappear. To ensure the safety of food products with high acid, such as citrus juice, pasteurization processing at a temperature between 60 and 100 °C is required. High-intensity heat treatment results in a bright yellowish color that is maintained during the storage period [10].

Kumar et al. (2005) reported that the use of a pulsed electric field with an intensity of 38 kV/cm in a duration of 24 microseconds and a frequency of 100 Hz had no significant effect on the content of ascorbic acid and the color of mango nectar, while the use of heat treatment in a hot water bath (temperature 95°C for 10 minutes) caused a decrease in the content of this vitamin and undesirable changes in the color of the product [11]. It was also observed that the treatment of broccoli juice using PEF (35 kV/cm, 100 Hz andμsμs 500-2000) resulted in greater retention of ascorbic acid content compared to conventional heat treatment at 90°C for 1 minute [12]. Vibio et al. (2019) reported that the PEF process was one of the best processing methods to preserve ascorbic acid in apple juice samples compared to fresh apple juice. Meanwhile, due to oxidative degradation reactions, the amount of ascorbic acid of the processed samples decreased significantly during storage. No significant changes were observed in the amount of ascorbic acid of PEF-processed juice and fresh sample [10].

In this research, the PEF system was designed and built, and the effect of time and different intensities of the pulsed electric field on the degradation rate of pectin methylesterase enzyme, ascorbic acid, turbidity and color during lemon juice processing was investigated.

2- Materials and method

2-1- Preparation of lemon juice

lemons (*Citrus auraniifolia*) after washing, it is cut into pieces and then using a juicer, it is drained and finally smoothed (mesh 170). Physical and chemical tests were performed immediately after sample processing.

2-2- Design and manufacture of PEF device

Figure 1-2 shows your diagram of a pulsed electric field processing system. In this device, a piston pump equipped with four syringes

4. Pectin methylesteras (PME)

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(volume 60 ml) and with the ability to adjust the flow rate was used to transfer the juice to the main chamber of the process. In the pump, a motor with a power of 12 watts was used to provide the driving force of the system. Electrical energy required by the systemIn this device, a frequency generator with the ability to change the voltage and pulse width is embedded. The voltage change in this device is done by a 500V-A variac whose input voltage is 220V single phase and its output can be changed from 0 to 300V. Variac is used as the power source of the device. Changing the frequency and pulse width is done using an electronic circuit with irfp460 power MOSFETs that are closed in the form of an H bridge. The output voltage from the variac alternately enters a diode bridge and is stored in the capacitor after being rectified. When this direct current enters the switching circuit, which consists of 12 MOSFETs, and the MOSFETs are turned off and on at the desired frequency, the current comes out as square pulses from the output of the device. Output pulses can be set as unipolar and bipolar. An AVR microprocessor is used in the electronic control system that adjusts the frequency, pulse width and number of pulses applied to the sample. Two electrodes made of stainless steel (314) with a length and thickness of 100 and 0.5 mm were used in the processing chamber of this device. Two electrodes are placed parallel to each other with a certain distance of 0.45 mm. The intensity of the electric field (E) is determined as the ratio of the electric potential difference (kV) for two electrodes to the distance (d). Therefore, by adjusting the output voltage of the device, a pulse electric field with intensities of 22.22, 33.33, 44.44 and 55.55 kV/cm was applied to the product in the time interval of 500, 571, 666, 800, 1000 microseconds. Immediately after PEF treatment, the samples were cooled in an ice water bath until reaching ambient temperature. The schematic view of the pulsed electric field device is shown in Figure 1.

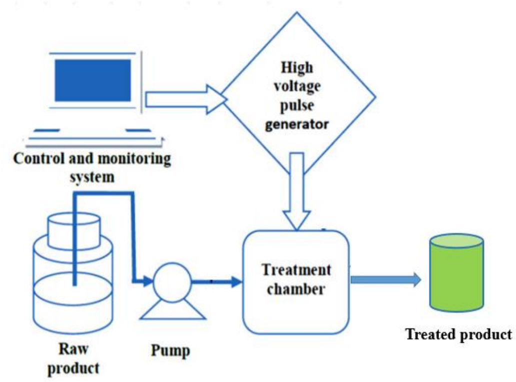

Fig 1 Schematic diagram of the system of PEF apparatus

2-3- Physicochemical tests

2-3-1- The amount of pectin methylesterase enzyme activity

In this study, to measure the activity of pectin methylesterase enzyme[5] The method provided by Kim Ball (1991) was used. In this method, the amount of acid produced is considered as a measure to measure the activity of this enzyme. First, in a 100 ml beaker, 20 ml of pectin salt solution (one liter solution containing 10 g of pectin and 15.3 g of sodium chloride salt) was added to 5 ml of processed lemon juice. Considering the effect of temperature on determining the activity of this enzyme, a 100 ml beaker containing the sample was placed inside a 250 ml beaker containing water during the test. Also, the temperature of the sample was set and maintained uniformly at 30°C using a magnetic stirrer. In order to increase the accuracy of the test, it is necessary to bring the pH of the sample to the neutral range using an alkaline solution (sodium hydroxide). After bringing the pH of the solution to about 7 using 2 normal soda, the pH of the solution was adjusted to 7.7 with the help of more dilute soda (0.05 normal). After that, 0.1 ml of 0.05 normal soda was added to the sample at once. The time required to produce acid due to enzyme activity on the carboxyl group in the structure of pectin and to neutralize the increase in pH caused by adding 0.1 ml of soda was recorded. By placing the pH return time as 7.7 in equation 1-3, the activity of this enzyme was calculated in terms

5. Pectin Methylesterase Activity (PEU)

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of (PEU/ml) [13].

$$PEU = \frac{N \times V_{NaOH}}{t \times V_{juice}}$$

In the above equation, N, V_{NaOH} ،t ،V_{juice} The normality of profit is the volume of added profit (ml), recorded time (minutes) and juice volume (ml), respectively.

2-3-2- The amount of ascorbic acid

In this study, iodine titration method (iodometry) was used to measure the amount of ascorbic acid. To prepare the iodine solution, first 5 grams of potassium iodide and 0.268 grams of potassium iodate were well dissolved in a 500 ml flask with 200 ml of distilled water, and then 30 ml of 3 M sulfuric acid was added to the resulting solution. At the end, the volume of the solution was increased to 500 ml with distilled water. In order to measure the amount of ascorbic acid based on this method, first 20 ml of lemon juice was mixed with 150 ml of distilled water. After adding one milliliter of 1% starch indicator solution, the resulting solution was titrated with iodine solution until a blue-black color appeared. The amount of mg of ascorbic acid in 100 ml of sample was calculated based on equation 2-3 [14].

= amount of ascorbic acid (mg per 100 ml of sample)
Volume of reagent consumed × 0.88

2-3-3- Cloudiness index (the degree of turbidity[6])

To measure cloudiness index (turbidity level), 5 ml of lemon juice was centrifuged (3000 rpm) for 10 minutes at ambient temperature (25°C). Then, the absorbance of the upper solution of the centrifuged sample was read in a spectrophotometer at a wavelength of 660 nm, which indicates the turbidity of the sample [15].

2-3-4- browning index

To check the color of fresh and processed lemon juice samples by image processing method[7] used. For this purpose, from a scanner[8] It was covered with completely black and thick fabric in order to create a completely isolated environment for taking pictures and to prevent errors due to changing the amount of ambient light and the angle of radiation. 10 ml of the sample was used in a Petri dish.[9] (with a height and diameter of one and nine centimeters, respectively) was transferred and photography was done according to the mentioned method. The images were saved with a resolution of 600 dpi and in JPEG format. After transferring the images to the computer, the L*, a* and b* values of the samples were extracted using Image J software version (1/47). Changes in browning index[10] (BI) was calculated according to equations 3 and 4 [15].

$$x = \frac{a^* + 1.75L^*}{5.64L^* + a^* - 3.012b^*}$$

$$BI = \frac{100 \times (x - 0.31)}{0.17}$$

2-3-5- Measurement of total annihilation

The polyphenols in lemon juice were measured colorimetrically with the first Ciocaltio reagent using a spectrophotometer. The phosphotungstic present in the reagent, which is a reducing acid with the chemical formula H3PW12O40, quickly regenerated the oxidized hydroxy group and at the end, a blue color was created, the maximum absorption of which is at the wavelength of 780 nm. In this test, gallic acid was used as a compound to measure polyphenols in lemon juice, in preparing a standard curve [16].

2-3-6- Sensory evaluation

The sensory evaluation of lemon juice samples was done by 10 sensory judges, 5 men and 5 women in the age group of 30-40 years, and before the test, the judges were given the necessary training and the samples were based on general acceptance, color, smell, sourness and Product appearance was evaluated on a 5-point hedonic scale from 1 dislike to 5 like. The samples of fresh juice and processed juice in a white disposable glass after processing with 3-digit codes along with a glass of water were provided to the group of evaluators. Sensory characteristics of lemon juice stored in glass bottles at 4°C and in the period of 45 and 90 days were investigated [17].

6. Turbidity
7. Image Processing
8. Scanner
9. Petri Dish
10. Browning Index

2-4- Statistical model of design and data analysis

Investigating the effect of the process on the physicochemical properties and kinetic parameters of the product, five levels of time and three levels of electric field intensity were used on the amount of pectin methylesterase enzyme activity, the amount of ascorbic acid, the clouding index and the amount of browning of the processed lemon juice. The data of the physicochemical tests were performed in three replicates using SAS software (version 1/9) in the form of completely random factorial design and with Duncan's test at a significance level of 95%. Data analysis and graphs were done with the help of Microsoft Excel 2013 software.

3. Results and Discussion

3-1- Physicochemical properties of fresh lemon juice

Some physicochemical characteristics of fresh lemon juice were measured in Table 1 [18].

Table 1 physico-chemical properties of fresh key lime juice

composition	content
pH	2.4
(kg/m^3) Density	1.46
Soluble Solid (gr/100)	7.8
Acidity (gr/100)	5.68
Ash (gr/100)	3.46

3-2- Inactivation of pectin methylesterase enzyme using PEF

Based on the results presented in Figure 2, it was found that during processing with PEF, the temperature of lemon juice increased due to the occurrence of ohmic heating and the application of a high-intensity pulsed electric field. Based on this, the temperature changes of the sample outlet after passing through the pulsed electric field were recorded. The lowest amount of temperature changes was observed in the pulsed electric field of 22.22 kV/cm and duration of 500 microseconds, 27 C and the highest amount of temperature changes was observed in the pulsed electric field of 55.55 kV/cm and duration of 1000 microseconds. In general, with the increase in the intensity of the pulsed electric field, the rate of temperature increase in PEF processing increased significantly ($p < 0.05$). Meanwhile, time changes had no significant effect on temperature increase ($p>0.05$). These results were observed with the research conducted during the processing of orange juice with PEF [20, 19].

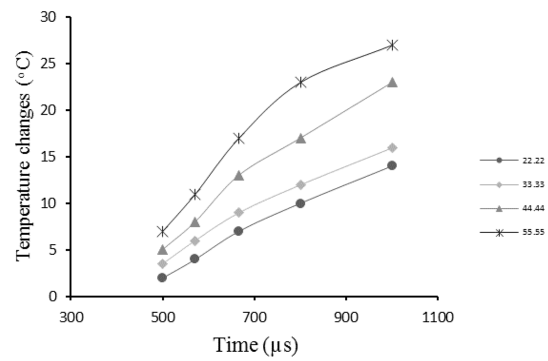

Fig 2 Temperature changes in PEF processing

The amount of pectin methylesterase enzyme activity[11] (PEU) in fresh lemon juice was 0.0013. The results of the statistical analysis showed that the application of pulsed electric fields with higher intensities had a significant effect on the degradation rate of PME enzyme ($p < 0.05$). The maximum temperature change of the sample was at the highest field intensity (55.55 kV/cm) and the process time was 1000 microseconds. The reason for the increase in temperature during the PEF process is attributed to the ohmic process. The increase in the temperature of the sample was consistent with the findings of Yeom et al. (2002) [19]. In his research, an increase in the temperature of the orange juice sample during the PEF process was reported to be 31°C. In addition, the output temperature of tangerine juice obtained from PEF processing at the intensity of the pulsed electric field of 25 kV/cm and the process time of 330 microseconds, C° 72 and the sample of orange juice processed with PEF at the field intensity of 23 kV/cm and time of 36 microseconds, C° 58 were reported [21]. In general, by increasing the field from 22.22 to 55.55 kV/cm at a constant processing time, the

11. Pectin-Esterase-Unit

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degradation rate of PME enzyme increased by 25% on average. Therefore, it can be said that there is a direct relationship between the intensity of the pulsed electric field and the destruction of the PME enzyme. In addition, with the increase in PEF application time on the sample, the degradation rate of PME enzyme increased ($p<0.05$). In general, it can be said that the lowest amount of degradation of PME enzyme was 22.1% related to the intensity of the field of 22.22 kV/cm and a time of 500 microseconds, and the maximum amount of degradation was observed in the electric field of 55.55 kV/cm and a process time of 1000 microseconds. It was 63.4 percent. Although PEF processing is considered a non-thermal technology, the increase in temperature during this process is considered as an important parameter and can play an important role in the inactivation of microorganisms, enzymes, as well as the destruction of unstable bioactive compounds in heat. Increasing temperature improves the inactivation of PME enzyme during PEF process. Most enzymes, including PME enzyme, are resistant and stable at room temperature during the PEF process. The amount of input energy and process time have a significant effect on the degradation rate of this enzyme. By applying an input energy of 44 megajoules per liter and a processing time of 8000 microseconds, inactivation of 93.8% of PME enzyme was observed in tomatoes. The highest temperature increase in this study was reported as 15 C] [7, 22, 15]. Similar results were observed by Martinez et al. (2007). Using a pulsed electric field of 35 kV/cm and a duration of 1500 microseconds (8 MJ/L) increased the temperature to 35°C and inactivated 77% of PME enzyme degradation [7, 23, 15]. The structural changes of enzymes after PEF process were first reported by Yeom et al. in 1999. 90% reduction of PME enzyme in orange juice under PEF treatment with an electric field intensity of 35 kV/cm in 59 microseconds was reported by Yeom et al. (2000) [19]. Rodrigo et al. (2003) by applying a pulsed electric field of 25 kV/cm for 340 microseconds to carrot-orange juice mixture reached 80% inactivation of PME enzyme [24] (Figure 3).

Inactivation of PME enzyme in different plant sources (orange, carrot, banana) was investigated after PEF treatment (total time 1.6 ms and electric field strength 19.1 kV/cm). Inactivation of PME enzyme in orange, tomato, carrot and banana was observed in 87, 87, 83 and 45% respectively [25]. In the inactivation of the enzyme, the first thing that happens is the unfolding of the protein structure. These changes are of covalent type that lead to conformational changes[12] It is in the special structure of protein. The secondary and tertiary structures of enzymes are stabilized by hydrogen bonds, whose change by electric pulse fields may lead to denaturation of the alpha-helix structure.[13] protein and as a result the disruption of the tetramer structure[14] becomes an enzyme; Many studies have indicated the destruction of alpha helical structure by PEF. There is also a direct relationship between loss of alpha helical structure and enzyme inactivation. The PEF process did not change the amino acid profile in grape juice, which indicates that PEF does not affect the primary structure of peptides and amino acids. Turfi et al. (2015) reported that no enzyme activity was observed during the storage period of fruit juice processed by the PEF process. In the PEF method, more active sites on the enzyme or a longer interaction of the enzyme with the substrate are observed [26, 20]. In addition, the pulses can lower the activation energy of the reaction. The most important effects of the PEF process on the protein structure include the movement of free electrons, ions, other charged compounds, the polarity of band movements, electrons in atoms, atoms in molecules, molecular rotation and the creation of momentary dipoles and finally the change of dielectric constant. In addition, the immediate increase in temperature during the pulse may lead to denaturation[15] PME enzyme helps. The synergy between heat and PEF, reported in a number of studies, is related to the increased mobility of charged groups at higher temperatures, which affects the electrostatic interactions and stability of proteins. In general, deactivation of the enzyme by heat treatment may be due to the denaturation of the enzyme, which is consistent with the results of

12. Conformational
13. Alpha Helical
14. Tetrameric
15. Denaturation

Krepfenber et al. (2006) [28, 27].

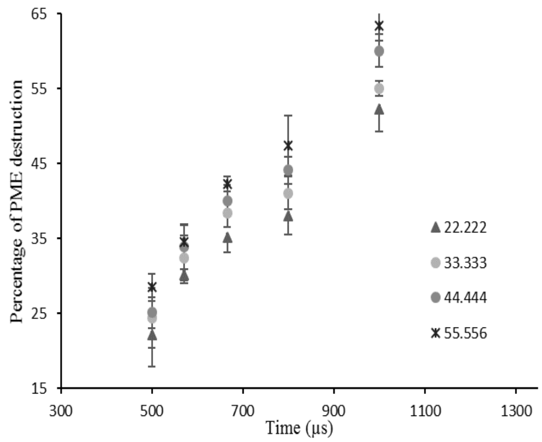

Fig 3 The effect of pulsed electric field and time on PME enzyme destruction in PEF process

3-2-2- The effect of PEF processing on the amount of ascorbic acid in lemon juice

In this study, the amount of ascorbic acid in fresh lemon juice was equal to 22.24 mg per 100 grams of extract. The effect of the intensity of the pulsed electric field on the amount of ascorbic acid changes during different processing times is shown in Figure 3-3. As the intensity of the field increased, the amount of ascorbic acid in the lemon juice sample decreased ($p<0.05$). In general, it can be said that with the increase in the intensity of the pulsed electric field in the fixed processing time, the degradation rate of ascorbic acid increased by 2.5%.

When the intensity of the pulsed electric field increases, the structure of ascorbic acid changes from the enol form to the keto form, which leads to an increase in the degradation rate of ascorbic acid [29 and 38]. 22.22 kV/cm and the duration was 500 microseconds, and the highest degree of degradation of the processed sample was at the electric field intensity of 55.55 kV/cm and the duration was 1000 microseconds. The effect of time on the degradation of ascorbic acid was statistically significant ($p<0.05$). In this research, by applying the highest electric field intensity of 55.55 kV/cm for 1000 microseconds, about 86% of ascorbic acid was preserved. These results were consistent with Ems et al. (2009). About 99.9% of the amount of ascorbic acid of watermelon juice processed with PEF was maintained at the intensity of the pulsed electric field of 35 kV/cm in the processing time of 50 microseconds and the frequency of 50 Hz, which with increasing the intensity of the pulsed electric field (frequency and pulse width) more than Ascorbic acid decreased by 50% [29].

The PEF process is one of the best non-thermal processing methods to preserve more ascorbic acid in fruit juices. Ascorbic acid is a heat-sensitive compound and is considered as an indicator of nutritional quality during food processing. If ascorbic acid is preserved in food, other nutrients will also be preserved [10].

In general, this shows the high thermal sensitivity of this compound and the importance of controlling the production process. High temperature leads to the loss of ascorbic acid. Because heat accelerates the oxidation process of ascorbic acid. The results of Elise Martins et al.'s research (2007) showed that 10% of ascorbic acid in the sample is destroyed during PEF processing, which was attributed to the increase in temperature during PEF processing due to ohmic heating. They reported the destruction of 1% of ascorbic acid at a field intensity of 35 kV/cm and a duration of 59 µs and an outlet temperature of 60 °C [9]. However, Evandlik et al. (2000) did not observe any change in the amount of ascorbic acid in apple juice processed with PEF.

In this study, the pulse electric field was 35 kV/cm and the processing time was 94 microseconds (output temperature 38 C) [30]. Such a similar result was reported in the researches of Min et al. (2003) [31]. Therefore, it is possible to observe the detrimental effect of temperature increase compared to process time reduction on the sensory and nutritional quality of fruit juice using the PEF process by applying the same pulsed electric field. In order to minimize the destruction of ascorbic acid in the PEF process, the outlet temperature is lower than 50°C [32].

The retention of ascorbic acid depends on the intensity of the electric field, process time, pulse frequency, and pulse width during the process. The lower the intensity of the field and the shorter the process time, the higher the retention of ascorbic acid. In addition, the bipolarity of the PEF process results in the retention of more ascorbic acid compared to the unipolarity of the

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process. The retention of more than 87.5% of ascorbic acid in orange juice and 84.3% for gazpacho in the intensity of the pulsed electric field of 35 kV/cm in the process duration of 1000 microsecond was reported [33]. By reducing the process time to 750 microseconds, 93% of ascorbic acid was preserved in orange juice processed with the intensity of the pulsed electric field of 35 kV/cm (temperature increase o 50 C) [34].

In general, the retention of more ascorbic acid can be attributed to the low pH in lemon juice. Because ascorbic acid has more resistance and stability in acidic conditions [35]. The degradation of ascorbic acid is influenced by compounds that participate in oxidation reactions. In addition, the reduction of ascorbic acid in fresh juices is also caused by oxidative enzyme reactions that are accelerated by ascorbate oxidase and peroxidase [36].

Many researches reported the use of PEF to process fruit juices as a new and effective method to preserve more of this vitamin compared to the conventional thermal method [37, 32, 9]. which is attributed to the lower temperature and short time of the PEF process compared to thermal processes [23 and 38].

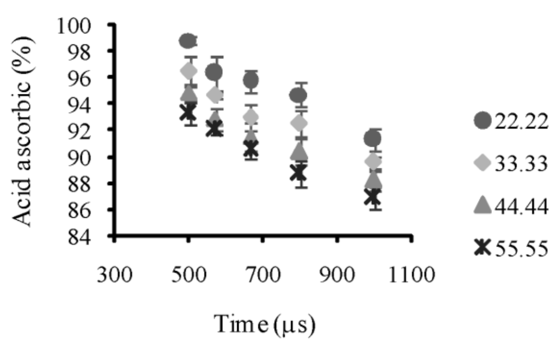


Fig 4 The effect of pulsed electric field and time on acid ascorbic content in PEF process

3-2-3- The effect of PEF processing on changes in cloudiness of lemon juice

The stability of the cloudy state plays an important role in the appearance and creating a good mouth feel. Turbidity positively affects the color and organoleptic properties of citrus juice. As shown in Figure 4-3. With the increase of pulsed electric fields, the turbidity of the sample increased ($p<0.05$). In addition, a direct relationship between processing time and turbidity level was observed ($p<0.05$).

By increasing the intensity of the field in the processing times (1000, 800, 666, 571, 500 microseconds), the amount of turbidity increased by 8% on average. These results were consistent with the research reported by Burridge (1997) [39]. Pectin is an acid-sugar derived polymer that has a significant effect on the cloudiness stability of fruit juice. The PME enzyme esterifies the methoxylated pectin, which causes the cloudiness of the juice to disappear. Therefore, a direct correlation between the degradation rate between PME enzyme and turbidity changes was observed in this research. Krak and Kroding (2006) reported that the methoxy groups of pectin molecules may be de-esterified by the enzyme in the presence of divalent cations such as calcium or magnesium and create cross-links with ions, which leads to the formation of a gel and, as a result, the cloudiness of the juice disappears. Therefore, the decrease in clouding stability is due to the activity of the PME enzyme and is less dependent on changes in particle size [40]. Similar results were reported by Reiner et al. (2009) [41]. Yeom et al. (2000) reported that citrus juices treated with PEF have turbidity stability comparable to heat pasteurized juices [9]. Therefore, it can be said that the use of high-intensity pulsed electric fields can have a significant effect in maintaining the turbidity of juice compared to the control sample.

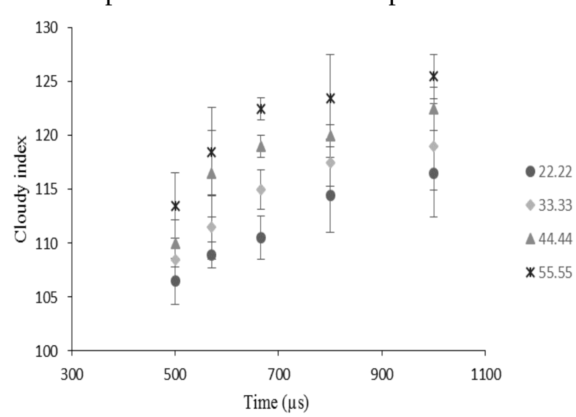


Fig 5 The effect of pulsed electric field and time on turbidity index in PEF process

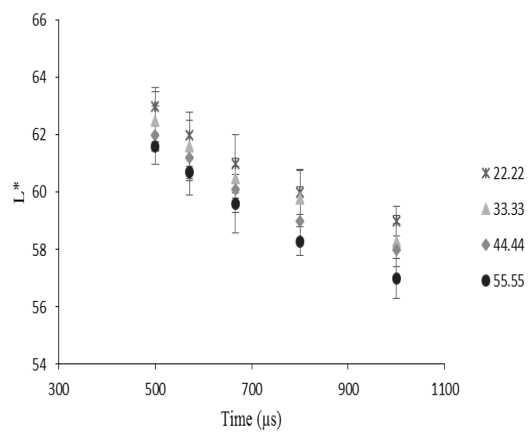
3-2-4- Changes in color factors

Figure 6(a) shows the effect of processing conditions on the color brightness of fruit juice. The statistical analysis of the results showed that the use of different intensities of the pulsed electric field had a significant effect on the color factors (L*, a*, b*) ($p<0.05$). In addition, as the process time increased, the brightness of the product decreased ($p<0.05$). The reason for the darkening of the sample can be attributed to the destruction of compounds such as pigments, ascorbic acid and the change in the turbidity of the sample. The amount of brightness index in the control sample 64/05 was reported that by applying high-intensity pulsed electric fields, the degradation rate of PME enzyme increased, and as a result, the amount of turbidity of the preserved fruit juice and the brightness of the product decreased. The highest amount of brightness decrease was in the processed sample with the pulsed electric field intensity of 55.55 kV/cm and duration of 1000 microseconds.

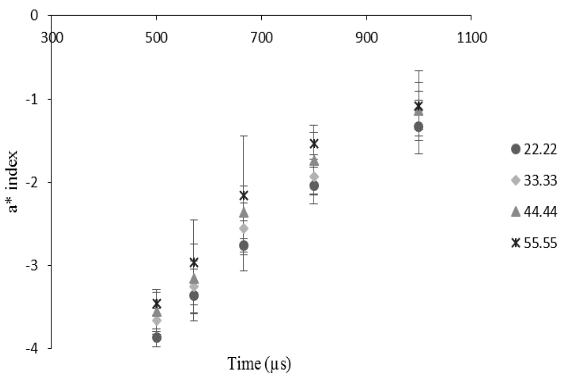
According to the increase in the amount of a* component, it can be reported that the color of lemon juice changed to red during longer processing ($p<0.05$). Analysis of variance as well as comparison of the average changes in the amount of a* of different treatments showed that the intensity of the electric field has a significant effect on this component ($p<0.05$). While the duration of electric pulse processing on this component was not statistically significant ($p<0.05$). In general, it can be said that the PEF process can be useful to a large extent in maintaining the appearance of the product similar to fresh lemon juice due to the short process time (Figure 4-5 b). In general, there are many studies that show no change in the color of samples processed with PEF, including grapes, lemons, tangerines, and orange juice [42, 43] Elise Martinez et al., 2006). The reason for the better preservation of color in the PEF process is the decrease in the formation of 5-hydroxymethyl-2-furfural. This compound is considered as an indicator of non-enzymatic browning reactions in fruit juices [31].

Figure 4-5 c shows the changes in the b* index of lemon juice color after the PEF process. By changing the intensity of the electric field from 22.22 to 55.55 kV/cm in different processing times, the b* index increased ($p<0.05$). In the constant processing time of 500 microseconds with the increase of the field from 22.22 to 33.33, 44.44 and 55.55, the amount of changes of this component compared to the control sample is 0.75%, 1.21%, 5.4% and 6.45% respectively. was observed. With increasing processing time, the amount of this component increased, which was not statistically significant ($p<0.05$). More redness was observed in products processed with PEF in tomato juice [31], orange juice [44] and tangerine juice [29].

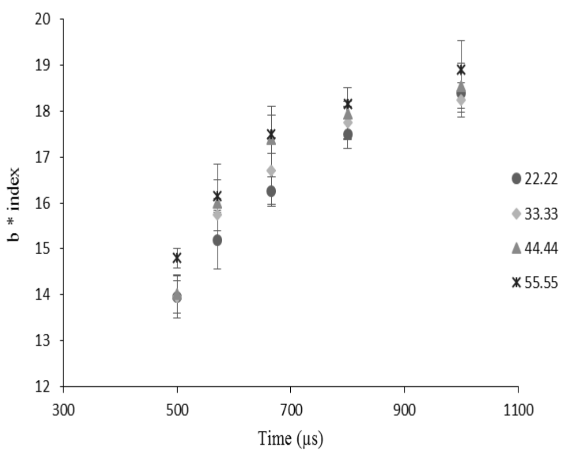
The results of this research were consistent with Qarchi and Barzegar, 2009 and Patras et al., 2009. The results of the research showed that pomegranate juice processed with PEF has a lower amount of brightness (darker color), the value of a* increased (red) and the value of b* (yellow) did not change [46 and 45]. Such similar results in the amount of color factors in sour cherry juice processed with PEF compared to the control sample L* (1.80 to 0.91), a* (2.75 to 3.11) and b* (85 0.0 to 0.56) and peach juice was observed [47 and 48].



(a)



(b)



(c)

Fig 7 The effect of pulsed electric field and time on L* (a), a* (b), b* (c) index in PEF process

3-2-4-1- browning index

As shown in Figure 8, the browning index increased with increasing pulsed electric field intensity and time ($p<0.05$). In all different intensities of the field, the value of L* decreased and the value of a*, b* increased, which indicates the browning and darkening of the color of the product. By increasing the process time from 500 to 571, 666, 800 and 1000 microseconds and at the field intensity of 22.22 kV/cm, the browning index was 3.80, 9.82, 14.76, 15.87 and 17.70, respectively. The percentage increased. The color characteristics of fruit juices are affected by non-enzymatic reactions and changes in natural pigments during product processing, including changes in temperature and time during the process [49]. The effect of increasing pulsed electric fields in fixed processing time was statistically significant. With the increase of the pulsed electric field from 22.22 to 33.33, 44.44 and 55.55 kV/cm, respectively, it was 3.80, 6.50, 13.29 and 15.59% in the processing time of 500 microseconds.

In general, the use of high-intensity pulsed electric fields increased the darkness of the product color compared to the control sample ($p<0.05$). These results were consistent with the researches of Victor et al. (2015) [50]. The highest amount of product color darkness in the processed sample was 41.86% compared to the control sample in the processing time of 1000 microseconds and in the intensity of the electric fields of 55.55 kV/cm. Therefore, it can be concluded that the use of high-intensity pulsed electric fields due to the increase in temperature caused by the ohmic process can destroy the pigments of the product and, as a result, darken the color of the product compared to the fresh sample. Such a similar result was observed in the tomato juice sample by Aguilo et al. (2008) [51]. The effect of the PEF process on the color of apple juice by Bai et al. (2013) showed that with the increase in the power of the pulsed electric field, the amount of brightness decreases and yellowness increases, which can be attributed to the deactivation of peroxidase and polyphena oxidase enzymes [52].

In general, the effect of PEF process on product color is complicated due to electroporation. Because electroporation causes intracellular material to leak out of the cell, so the activity of some enzymes increases. In addition, the application of PEF leads to the formation of reactive oxygen species[16] It can affect the optical properties of the product. PEF process prevents product color change by deactivating enzymes [53 and 54]. Meanwhile, decreasing the amount of illumination by increasing the intensity of the pulsed electric field and the time of the process leads to the release of more polyphena oxidase enzyme after electroporation [55].

16. Reactive Oxygen Species (ROS)

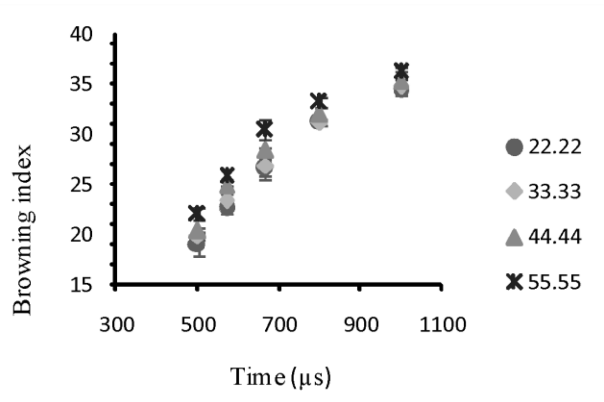


Fig 8 The effect of pulsed electric field and time on browning index in PEF process

3-2-5- Phenolic compounds

The amount of phenolic compounds in the control sample was 221.6 mg per liter. By increasing the intensity of the pulsed electric field and the duration of PEF processing, the amount of phenolic compounds decreased significantly ($p<0.05$) (Figure 9). Phenols have been reported as secondary metabolites in plants, which play an important role in creating flavor and color in fruit juices. Phenolic compounds and ascorbic acid are the most important factors in the antioxidant activity of lemon juice. Many studies have proven the direct relationship between total phenolic content and antioxidant activity. With the increase in the intensity of the pulsed electric field, the amount of electroporation increases and a larger amount of phenolic compounds are released due to the destruction of the membrane; However, due to the increase in the output temperature of the PEF process caused by ohmic heating, the amount of phenolic compounds decreased. The highest rate of degradation of phenolic compounds was 9.5% in the sample processed at the pulsed electric field intensity of 55.55 kV/cm and duration of 1000 microseconds compared to the control sample. These studies were in accordance with Zhang et al. (1995) [56]. In general, the effect of the PEF process on the amount of antioxidant compounds in the sample depends on the control of the process conditions. Guo et al. (2014) reported that the amount of phenolic compounds processed with PEF in pomegranate juice under conditions of 35-38 kV/cm and a duration of 281 microseconds at a temperature of 55°C does not change [57]. In addition, the amount of antioxidant compounds increased in watermelon juice processed with PEF by increasing the intensity of the pulsed electric field from 25 to 35 kV/cm for a duration of 1050 microseconds [34]. In general, the PEF processing method as a new non-thermal method significantly preserves phenolic compounds compared to the thermal method. Application of PEF process leads to 18% reduction of phenolic compounds in Cheshalu water[17] while the thermal process caused a 42% drop of these compounds in Cheshalu water [58].

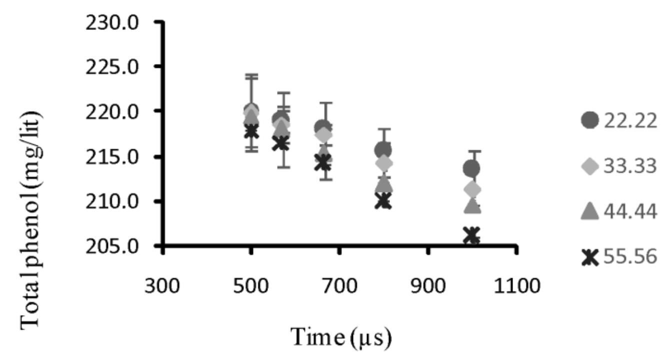


Fig 9 The effect of pulsed electric field and time on Total phenol in PEF process

3-2-5- Sensory evaluation

With the increase of pulsed electric field, color and process time of samples processed with PEF had a lower score compared to the control sample (Figure 10). The color of samples processed with PEF had a greater tendency to yellow in the storage period of 45 and 90 days compared to the control sample ($p < 0.05$). Processing with PEF did not have a significant effect on the smell and sourness of the sample, which indicates the preservation of aromatic compounds of lemon, including terpenes, hydrocarbons, ketones, and aldehydes during processing with PEF ($p<0.05$). The taste of samples processed with PEF in different intensities of the pulsed electric field and process time compared to the control sample had no statistically significant effect ($p<0.05$). During the storage period of samples processed with PEF, no bitter taste was reported in the

17. Longan juice

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samples, which is due to the destruction of enzymes that cause bitter taste in this product during processing with PEF. In general, the overall acceptance of the product processed with PEF was acceptable compared to the control sample in the storage period of 90 days. These results are consistent with Turkmeni et al. (2011). In general, the sensory and visual characteristics of the sample processed with PEF were very similar to the fresh sample [59].

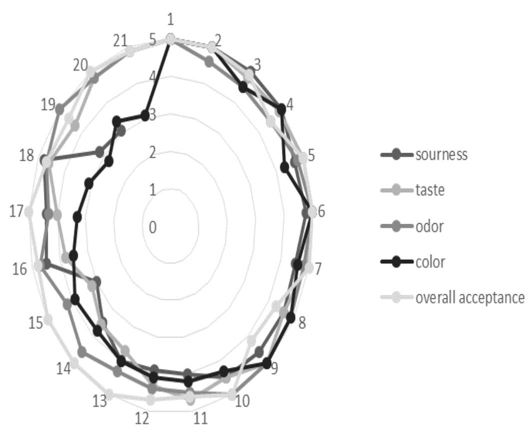

Fig 10 The effect of pulsed electric field and time on sensory evaluation in PEF process

4 - Conclusion

In recent years, the demand for good quality natural food products and food products with minimal processing and high nutritional value has increased. Pulsed electric fields is a non-thermal preservation technology that has been considered in the food industry due to the prevention of adverse changes. Among the foods in which it is very important to maintain the organoleptic characteristics. Due to the short duration of the pulses, little heat is generated in the foodstuff, and thus, the nutrients, vitamins, texture and aroma, as well as the microstructure of the foodstuff, compared to the samples that have been subjected to the thermal process It can be preserved better. Therefore, in order to maintain the quality characteristics of food, it is superior to thermal methods.

In general, by increasing the field from 22.22 to 55.55 kV/cm at a constant processing time, the degradation rate of PME enzyme increased by 25% on average. Therefore, it can be said that there is a direct relationship between the intensity of the pulsed electric field and the destruction of the PME enzyme. In addition, with the increase in PEF application time on the sample, the amount of PME enzyme degradation increased ($p<0.05$). As the intensity of the pulsed electric field increased, the percentage of ascorbic acid degradation increased. Applying the highest electric field intensity of 55.55 kV/cm for 1000 microseconds preserved 86% of ascorbic acid. With the increase of the field intensity and the time of the process, the browning index increased ($p<0.05$). In general, it can be said that using the non-thermal method of pulsed electric field to process acidic products and destroy enzymes is considered as a promising approach due to the preservation of quality properties such as ascorbic acid and phenolic compounds of the product compared to common thermal processes.

5-Resources

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مقاله علمی_پژوهشی

طراحی و ساخت سیستم فرآوری آب‌لیمو توسط میدان الکتریکی پالسی و ارزیابی ویژگی‌های کیفی آن

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چکیده

آب‌لیمو فرآورده غیر تخمیری با اسیدیته بالا و منبع غنی از اسید آسکوربیک و ترکیبات فنلی می‌باشد که از فعالیت رادیکال‌های آزاد در بدن جلوگیری می‌کند. امروزه به‌منظور حفظ کیفیت محصول از روش‌های نوین غیرحرارتی جهت فرآوری ماده غذایی استفاده می‌شود. هدف از این پژوهش طراحی و ساخت سیستم مداوم میدان الکتریکی پالسی (PEF) و تأثیر آن بر میزان تخریب آنزیم پکتین متیل استراز و محتوای اسید آسکوربیک و ترکیبات فنلی، اندیس ابری شدن و قهوه‌ای شدن وخصوصیات حسی در آب‌لیمو می‌باشد. در این پژوهش موج مربعی شکل دوقطبی به عرض ۱۰ میکروثانیه و فرکانس ثابت ۱۰۰۰هرتز مورد استفاده قرار گرفت. در محفظه فرآوری این دستگاه از دو الکترود از جنس استیل ضدزنگ به طول و ضخامت به ترتیب ۱۰۰ و ۰/۵ میلی‌متر استفاده شد. آب‌لیمو در مدت ۵۰۰، ۵۷۱، ۶۶۶، ۸۰۰، ۱۰۰۰ میکروثانیه تحت فرآیند PEF با شدت میدان الکتریکی پالسی ۲۲/۲۲، ۳۳/۳۳، ۴۴/۴۴ و ۵۵/۵۵ کیلوولت بر سانتی‌متر قرار گرفت. به‌طورکلی با افزایش میدان از ۲۲/۲۲ به ۵۵/۵۵ کیلوولت بر سانتی‌متر در یک‌زمان ثابت فرآوری میزان تخریب آنزیم PME به‌طور میانگین ۲۵ درصد افزایش یافت. اعمال بالاترین شدت میدان الکتریکی پالسی ۵۵/۵۵ کیلوولت بر سانتی‌متر در مدت‌زمان ۱۰۰۰ میکروثانیه موجب تخریب ۱۴ درصدی اسید آسکوربیک در مقایسه با نمونه تازه شد. با افزایش میدان الکتریکی پالسی میزان کدورت نمونه و اندیس قهوه‌ای شدن افزایش یافت. استفاده از میدان الکتریکی پالسی با شدت بالا موجب افزایش میزان تخریب آنزیم PME و حفظ کدورت در محصول فرآوری شده در مقایسه با نمونه تازه شد. حفظ خصوصیات حسی آب‌لیمو فرآوری شده با PEF در مدت‌زمان نگه‌داری نشان داد که استفاده از روش غیرحرارتی میدان الکتریکی پالسی می‌تواند به‌عنوان یک رویکرد امیدوارکننده در فرآوری محصولات غذایی باشد.