

# Nutritional Quantification and Shelf Life Analysis of Non-thermal Processed Coconut Juice

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**Abstract.** Nonthermal processing methods are attracted by many food and beverage industry because it can kill the microbial contamination under mild temperatures used in thermal processing; They can sustain flavours, essential nutrients, and vitamins undergo minimal changes. The objective of this research was to evaluate nonthermal processing as the high current impulse generator (HCIG) and investigate the reduction the contaminated microorganism in coconut juice under the batch and continuous treatment of HCIG. The physical, biochemical and nutritional changes of treated coconut juice were also investigated. The application of the direct electricity through cathode of high current impulse generator (HCIG) in 1,170 chambers contained the contaminated coconut juice. Significantly reduction both *Saccharomyces cerevisiae* and *Escherichia coli* as 5-log were found when treatments were applied with impulses at 5.17 kA. Comparison of nutritional value of non-thermal processes before and after high current impulse was showed no significant differences between main nutritional values and free amino acid. For the continuous HCIG treatment under the treatment of 5.17 kA current with 9, 15 and 30 pulses with 5 L coconut juice at the flow rate of 1 L/min, results from initial concentration at  $1.41 \times 10^5$  CFU/mL showed that *S. cerevisiae* reductions were found 78%, 66% and 96% as increasing number of pulses as 9, 15 and 30 pulses, respectively. The increment of microbial reduction with the increasing number of pulses was also detected as 78 %, 82 % and 96 % from  $1.11 \times 10^5$  CFU/ml *E. coli*. Results revealed that the microbial reduction with HCIG under batch treatment were successful preserved the nutritional components of the coconut juice without significant physicochemical changes.

## 1 Introduction

Young coconut (*Cocos nucifera* L.) is one of the tropical region fruit that is recognized as global natural product as a nutritious and beneficial health beverage. It is cultivated in Thailand mostly central and southern part and well recognized as juice as a refreshing for sport drinker because of it acts as electrolytes from some minerals. Its juice has an excellent nutritional composition such as sugars, vitamins, minerals, amino acids and phytohormone leading to gaining the attention of plant industry, biotechnology and biomedical fields [1-3]. Apart from 94% water, coconut juice contains a variety of inorganic ions that contribute to the therapeutic value and the rich sources of vitamins, B1, B2, B3, B5, B6, B7 and B9 [4-5].

Nonthermal process as the pulsed electric field (PEF) treatment has the potential to be taken up as an alternative to processing of value-added food products. This treatment involves applying a short burst of high voltage to foods between two electrodes, and can be carried out at ambient or at refrigeration temperatures. The application of PEF has proved capable of increasing the shelf-life of various liquid foods, extending product shelf life and retaining

their physical, nutritional, and sensory qualities [6]. The inactivation of pathogenic or sporulated microorganisms or reduction of their growth under controlled conditions have been achieved and reported over the decade. Among the novel nonthermal processing technologies include pulsed or radio frequency electric fields (PEF/RFEF), ultraviolet light (UV), ultrasound (US), pulsed light (PL), high pressure processing (HPP), ionizing radiation, dense phase carbon dioxide (DPCO<sub>2</sub>) and ozone, the pulse electric field is considered as the most practical and promising technology to meet the pasteurization standards [7-11].

Previously, application of pulsed electric fields (PEF) in combination with mild thermal treatment was studied to extend the shelf life of whole milk, durian juice, mangosteen juice, coconut juice [12, 13]. Many studies indicated that when microbial cells were exposed to high voltage PEF, dielectrically breakdown of cell membrane occurred [14]. There are many factors that affect the microbial inactivation with PEF process factors (electric field intensity, pulse width, treatment time and temperature and pulse wave shapes), microbial entity factors (type, concentration and growth stage of microorganism) and media factors (pH, antimicrobials

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and ionic compounds, conductivity and medium ionic strength).

Therefore, the treatment chamber design is important in optimizing the inactivation of microorganisms. Pasteurization of coconut juice is thermally processed using ultra high temperature (UHT) technology. However, coconut juice loses its delicate fresh flavor and some of its nutrients during heating. A nonthermal process is desirable to protect the fresh flavor and nutrient content of coconut juice, which would increase marketability of this healthy drink and availability to consumers throughout the world.

This study evaluated the effects of pulsed electrical field treatment on physical and chemical quality of a coconut juice beverage. For nonthermal processes, there were not reported the completely reduction of contaminated microorganism. Therefore, the approach of this research was to evaluate high current impulse generator (HCIG) application on young coconut juice and investigate the influential parameters to completely reduction of the microorganisms under the batch and continuous treatment. The physical, biochemical and nutritional changes of treated coconut juice were also reported.

## 2 Materials and Methods

### 2.1 Raw materials

Coconut was purchased from a commercial farm (Nakornpatum, Thailand) and directly opened in the laboratory with all sterilized glass and container equipment. The preliminary analysis of microbial contamination from local market of young coconut juice by the standard biochemical tests were done and identified two microorganisms as *Saccharomyces cerevisiae* and *Escherichia coli*. The analyses were performed in triplicate. Microbiological media was obtained from Himedia, India. The treatment of contaminated coconut juice was prepared to evaluate the potential processing of the construct high pulse electrical field by adding *Escherichia coli* TISTR 780 and *Saccharomyces cerevisiae* TISTR 5092 in newly open young green coconut (no contamination with any microorganism). This contaminated condition was adding these two strains from the Thailand Institute of Scientific and Technological Research for using as the model organisms in this experiment.

### 2.2 Batch treatment of high current impulse generator

The high current impulse generator was carried out in an electric laboratory at Department of Electrical Engineering, Faculty of Engineering, King Mongkut's University of Technology North Bangkok, Thailand. This nonthermal process consisted of high voltage power source, an energy storage capacity blank, a charging current limiting resistor, a switch to discharge energy from capacitor across from coconut juice and a 1,170 mL treatment chamber. An oscilloscope was used to observe the pulse waveform. The power source, a high voltage DC

generator, converted from utility line (220 V) to high voltage AC, then rectified to high voltage DC. A current impulse generator model for the microbial reduction in coconut juice was tested according to petty patent no.1503000462 [15].

Energy from power source was stored in the capacitor and discharged through the treatment chamber to generate an electric field in the coconut juice. The maximum voltage across the capacitor was equal to the voltage across the generator. The construction of HCIG and the investigation of the reduction the contaminated microorganism in coconut juice under the batch was carried out. The application of the direct electricity through cathode of HCIG in 1,170 mL chamber contained the treated coconut juice with  $9.4 \times 10^4$  CFU/mL *Saccharomyces cerevisiae* TISTR 5092 and  $8.6 \times 10^4$  CFU/mL *Escherichia coli* TISTR 780 with the average current of 3.57, 4.37 and 5.17 kA with 3, 6, and 9 pulses was carried out 22 °C. The treated coconut juice was also investigated by sampling in two sections of the chamber (top and middle).

### 2.3 Continuous treatment of high current impulse generator

5 L of coconut juice was prepared by adding the mixture of  $1.41 \times 10^5$  CFU/mL *S. cerevisiae* TISTR 5092 and  $1.1 \times 10^5$  CFU/ml *E. coli* TISTR 780 before HCIG application. For the continuous treatments of HCIG, the experiment was carried out under 5.17 kA with 9, 15 and 30 pulses by connecting 5 L of contaminated coconut juice to pump at the flow rate of 1 L/min. Samples were collected at the end of process for checking for number of microorganism changes before and after the treatment.

### 2.4 Pasteurization

1,170 mL treated coconut juice was processed with pasteurization in waterbath at 90°C for 15 min before use.

### 2.5 Physical, chemical and microbiological analysis of coconut juice

#### 2.5.1 The total soluble solid and pH

The total soluble solids content of the young coconut juice was measured using the hand held refractometer according to AOAC, (1990) [16] and the values were expressed as degree °Brix. The pH was determined using a Digital pH meter.

#### 2.5.2 Mineral detection and amino acid profile

Vitamin C (L-ascorbic acid) was detected using High Performance Liquid Chromatography (HPLC) [17] and Vitamin B2 (Riboflavin) was analyzed based on standard method [18]. The analysis of fresh and processed coconut juice samples was determined the mineral contents by using inductively coupled plasma mass spectroscopy. Free amino acids were analyzed by HPLC according to the

standard method [19]. The browning intensity of sugar was detected by spectrophotometer at 420 nm.

### 2.5.3 Microbiological analysis

Treated coconut juice and a control (untreated) young coconut juice were subjected to microbiological analysis. The total viable numbers of two bacteria expressed as CFU/mL were determined by plate count method using yeast malt agar for *S. cerevisiae* and Eosin methylene blue agar for *E. coli*. A control (fresh young coconut), treated by HCIG for inactivation experiment were collected in each setting in two sections of the chamber (top, middle) in order to confirm the distribution of microorganism and were kept on ice before serially dilution to plate count method.

### 2.5.4 Physicochemical of shelf life analysis

Samples of treated HCIG were kept at 4°C and 30°C over four weeks storage time for monitoring the changes in total soluble solid pH and browning reaction. Control from fresh soluble solid, pH and browning reaction was tested. Control from fresh coconut juice samples were also carried out in the same temperature.

### 2.6 Sensory analysis

Samples of fresh young green coconut juice, coconut juice after treatment with batch HCIG with 5.17 kA, 9 pulses without spike with microbe and pasteurization were collected in the sterile bottles and stored at 4°C before sensory analysis. Sensor evaluation of control and processed coconut juice was assessed by using 30 untrained panelists at 25°C. The acceptability was evaluated in seven attributes like appearance, color, taste, aftertaste and overall acceptability by using a 9-point hedonic scale from 1 (dislike extremely) over 5 (neither like nor dislike) to 9 (like extremely). The fresh and processed coconut juice were prepared one day before sensory evaluation and kept in 4°C. The test samples were labeled with three-digit number code selected from a random number table and served three samples to each panelist. Panelists were asked to rinse their mouths with water between samples.

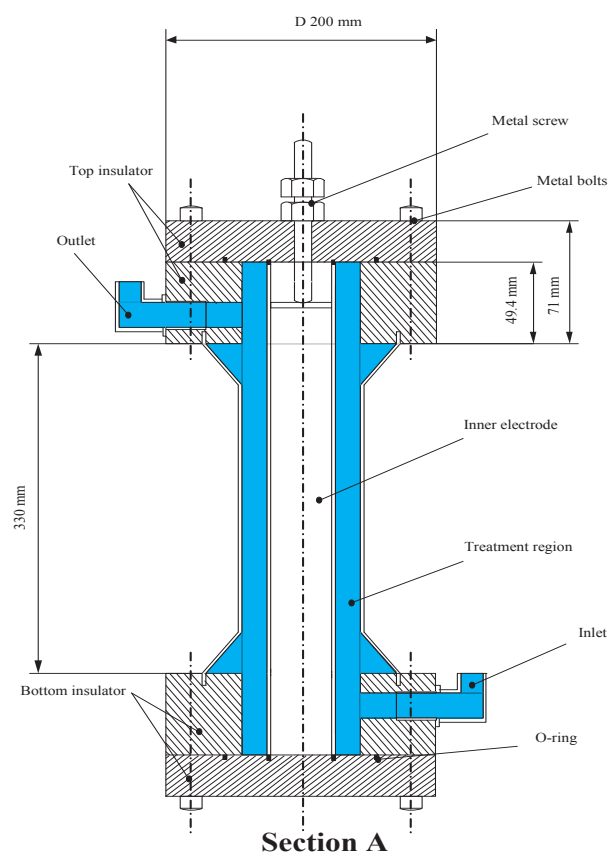
### 2.7 Statistical analysis

Experiments were performed using a Complete Randomized Design. All experiments were done in triplicate. The significant differences in mean values were analyzed by one-way ANOVA using LSD for post-hoc comparison using SPSS version 12.0.

## 3 Results and discussion

### 3.1 High current impulse generator effect on batch and continuous treatment

A systematic run by the application on the high current impulse generator was applied with control and treated coconut juice with mixed microorganism. The data of current, pulse shape and voltage was recorded with a digital oscilloscope. The set up treatment of coconut juice treatment in batch and continuous was illustrated in Fig. 1 (a, b) and tabulated in Table 1. The application of the direct electricity through cathode of high current impulse generator (HCIG) in 1,170 chamber contained the contaminated coconut juice with  $9.4 \times 10^4$  CFU/mL of *S. cerevisiae* and  $8.6 \times 10^4$  CFU/mL of *E. coli* with the average current of 3.57, 4.37 and 5.17 kA with 3, 6, and 9 pulses was carried out at 22°C. The illustrations of the setup was shown in Fig. 1. Results found that there was no changes in temperature in inlet and outlet at 22°C of all treatments (Table 1).



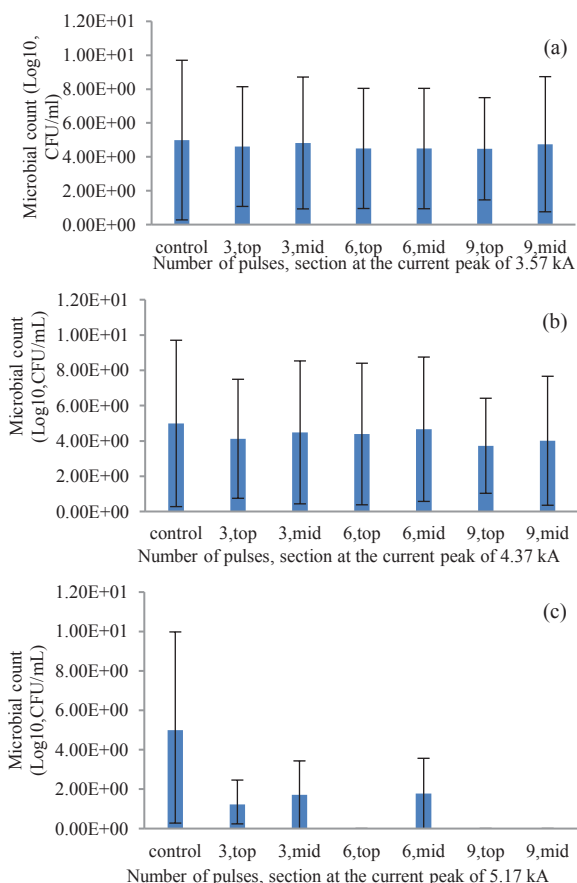
**Fig. 1.** An illustration of a cross section of high current impulse generator (HCIG) chamber.

Results showed that  $9.4 \times 10^4$  CFU/mL *S. cerevisiae* and  $8.6 \times 10^4$  CFU/mL *E. coli* with the average current of 3.57 kA with 3, 6, and 9 pulses with 100 V was of 22°C (Fig. 2 and 3). *S. cerevisiae* reductions were found 59%, 68% and 69% as increasing numbers of pulses, respectively (Fig. 3a). When the average current was increased to 4.37 kA with the increasing pulses, the reduction was found 86, 75 and 95% in the top section but only 69, 53 and 90% found in the middle section. The investigation showed the average reduction was found 78% of the initial treated (control). The top section revealed more reduction than the middle section (Fig. 2b). Under 4.37 kA applied with 9 pulses, the top section found the 10 log reduction. For the HCIG treatment at

5.17 (Fig. 2c), there was no detection of *S. cerevisiae* found in any section from 3, 6, and 9 pulses.

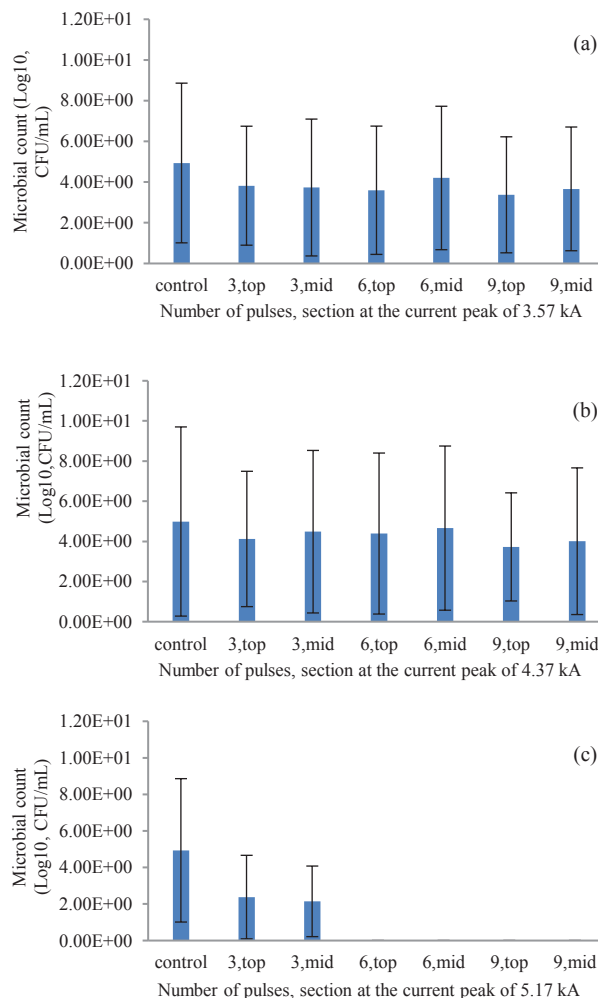
**Table 1.** Treatment parameters for a high current impulse generator for a batch coconut water processing.

Maximum current [kA]	Number of pulses [pulses]	Voltage [V]	Inlet temperature [°C]	Outlet temperature [°C]
3.68	3	100	22	22
3.52	6		22	22
3.52	9		22	22
4.40	3	125	21	21
4.40	6		22	22
4.32	9		22	22
5.12	3	150	19	19
5.12	6		19	19
5.28	9		18	18



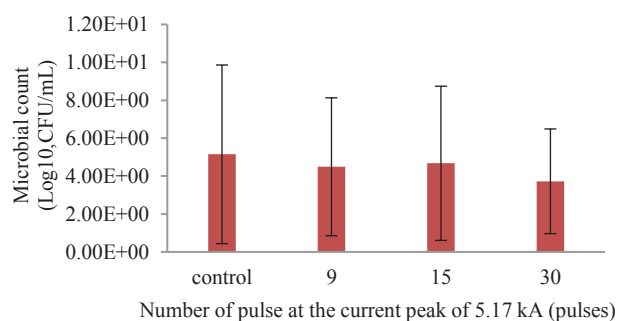
**Fig. 2.** Reduction of *S. cerevisiae* under the high current impulse generator for a batch coconut water processing at the average of maximum current (a) 3.57 kA, (b) 4.37 kA and (c) 5.17 kA.

For *E.coli* treatment, the average current of 3.57 kA with 3, 6, and 9 pluses with 100 V, the top sections showed a higher reduction of this pathogen by 20%. The observation found 90, 83 and 92% reduction from initial treated *E.coli* in coconut juice while in the middle section found 70, 60 and 87% with 3, 6, and 9 pluses (Fig. 3a, b). Significant reduction in both *S. cerevisiae* and *E.coli* as 5-log was found when treatments were applied with impulses at 5.17 kA when compared with 3.57 and 4.37 kA with  $p=0.01$  and  $p < 0.001$ , respectively.

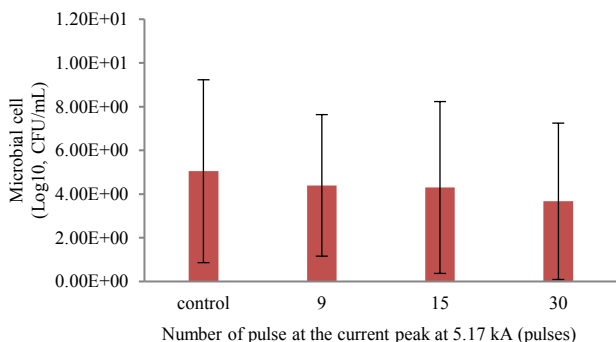


**Fig. 3.** Reduction of *E. coli* under the high current impulse generator for a batch coconut water processing at the average of maximum current (a) 3.57 kA, (b) 4.37 kA and (c) 5.17kA

Due to the successful inactivation by batch HCIG treatment at 5.17 kA, we ran the continuous HCIG under the treatment of 5.17 kA current with 9, 15 and 30 pulses with 5 L coconut juice at the flow rate of 1 L/min. The application of impulse high current was applied from the cathode. It was found that *S. cerevisiae* was reduced by 5.04, 4.97 and 5.13 log phase or 86, 75 and 95% (Fig. 4), respectively. For *E. coli*, the reduction from the increasing number of pluses found 4.93, 4.95 and 5.02 log phase or 78, 82 and 96, respectively (Fig. 5) in those order.

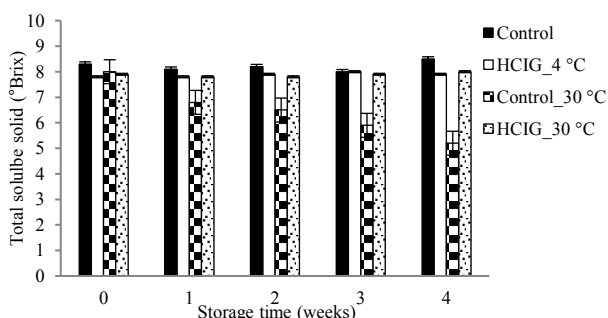


**Fig. 4.** Reduction of *S. cerevisiae* under continuous of the high current impulse generator for a batch coconut juice processing at the average of maximum current of 5.17 kA.

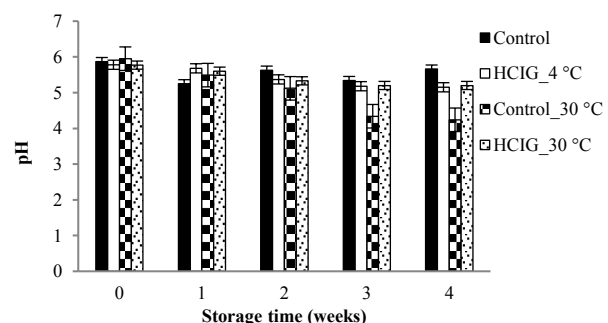


**Fig. 5.** Reduction of *E. coli* under continuous of the high current impulse generator for a batch coconut juice processing at the average of maximum current of 5.17 kA.

Under batch and continuous treatment with 1,170 mL contaminated coconut juice with maximum electrical intensity 63 kV/cm, 10 pulses with 10,000 pF of the capacitance with capacity (condition A1), *S. cerevisiae* was reduced by approximately 97% in three sections (top, middle and bottom) from initial contamination of  $1.41 \times 10^5$  CFU/mL. When the capacitance increased to 30,000 pF (condition A2), results found 99% reduction in the same microorganism (Fig. 6). In both conditions (A1 and A2), 5-log microbial reduction were found. For *E. coli*, there was no significant difference in treatments, 5-log reduction when compared between 10,000 pF with 30,000 pF (Fig. 6 and 7).



**Fig. 6.** Total soluble solid changes over shelf life of 4 weeks.

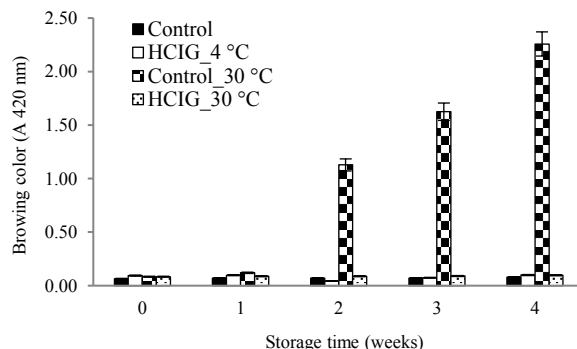


**Fig. 7.** pH changes over shelf life of 4 weeks.

### 3.2 Physicochemical changes in coconut juice

The result on physicochemical changes on coconut juice passed through HCIG by batch processing at the average of maximum current at 5.17 kA was shown in Table 3. The results showed most of nutritional values were not

changed. All amino acid detected in before and after HCIG process showed the same amount. There were only small changes in minerals as chloride, zinc, potassium and phosphorous by 6.43, 5.56, 2.67 and 0.30% found in coconut juice after treatment. However, a noticeable change in vitamin B2 was found about 48.14%. Table 3. and Fig. 8 showed no browning occurred before and after treatment.



**Fig. 8.** Browning color changes over shelf life of 4 weeks.

**Table 3.** Nutritional quantification of coconut juice with batch of high current impulse generator at 5.17 kA and 9 pulses.

Composition	High current impulse generator	
	Before	After
Carbohydrate (g/mL)	0.98	0.98
Protein (mg/mL)	1.8	1.8
Lipid (g/mL)	0.014	0.014
Vitamin C (mg/mL)	0.019	0.019
pH	5.09	5.09
Browning (Absorbance 420 nm, AU)	0.025	0.025
Calcium (mg/100g)	16.02	16.03
Magnesium (mg/100g)	7.30	7.31
Sodium (mg/100g)	6.94	6.82
Chloride (mg/L)	1181	1105
Zinc (mg/100g)	0.18	0.17
Copper (mg/100g)	<0.10	<0.10
Manganese (mg/100g)	<0.10	<0.10
Potassium (mg/100g)	224.72	218.36
Phosphorous (mg/100g)	5.32	5.30
Vitamin B2 (mg/100g)	1.35	0.70
Alanine (mg/100g)	3	3
Arginine (mg/100g)	5	5
Aspartic acid (mg/100g)	0	0
Cysteine (mg/100g)	0	0
Glutamic acid (mg/100g)	1	1
Glycine (mg/100g)	1	1
Histidine (mg/100g)	0	0
Isoleucine (mg/100g)	0	0
Leucine (mg/100g)	0	0
Lysine (mg/100g)	1	1
Methionine (mg/100g)	0	0
Phenylalanine (mg/100g)	0	0
Proline (mg/100g)	3	3
Serine (mg/100g)	0	0
Threonine (mg/100g)	0	0
Tryptophan (mg/100g)	0	0
Tyrosine (mg/100g)	0	0
Valine (mg/100g)	0	0

### 3.3 Sensory Evaluation

Table 4 showed the values of sensory attributes among fresh coconut, conventional pasteurization process and HCIG (5.17 kA, 9 pulses). Results found that there was no significantly difference in all sensory analysis of fresh and HCIG. However, all the panelists detected significantly differences in appearance, color and taste of pasteurization versus fresh and HCIG.

**Table 4.** Sensory evaluation score with 9-point hedonic scale from batch treatment of coconut juice at 5.17kA with 9 pulses.

Coconut process	Appearance	Color	Taste	After taste	Total acceptability
Fresh	6.66±0.96 <sup>a</sup>	6.86 ±0.80 <sup>a</sup>	8.70 ±1.08 <sup>a</sup>	6.93 ±1.05 <sup>a</sup>	7.13±0.85 <sup>a</sup>
Pasteurization	5.70±1.88 <sup>b</sup>	4.66 ±0.88 <sup>b</sup>	6.76 ±0.88 <sup>b</sup>	6.10 ±1.34 <sup>a</sup>	6.46±1.44 <sup>a</sup>
HCIG	6.41±1.86 <sup>a</sup>	6.66 ±0.88 <sup>a</sup>	7.90 ±0.99 <sup>a</sup>	6.56 ±1.83 <sup>a</sup>	7.76±1.88 <sup>a</sup>

Samples of treated HCIG were kept at 4°C and 30°C over four weeks of storage time for monitoring the changes in total soluble solid pH and browning reaction as the comparison of shelf life shown in Fig. 6-8. Similar trend in the reduction of total soluble sugar and pH reduction in to acidity side was found in Fig. 6 and 7. Furthermore, there was significant increasing in browning reaction found in second week to the fourth week of shelf life period. Results suggested that HCIG could sustain the changes in physicochemical attributes if the treated coconut juice was kept in 4°C.

Results revealed that the microbial reduction with batch HCIG treatment was successful reduction the microbial contamination at 5.17 kA with 9 pulses and preservation of the nutritional components of the coconut juice without significant physiochemical changes resulting the prolong of shelf life of processed coconut juice. Our results were in agreement with Bourneowa, 2015 who found that the set up pulse electrical field (PEF) was significantly effective at 20-40 kV for microbial reduction in the coconut water, the durian juice and the mangos teen juice, except for orange juice. The PEF-treated coconut water was also effective to reduce the microbial load of 0.96 and 1.48 log CFU/mL at 20 and 40 kV, respectively [13]. Our results were in accordance with the Kathiravan, 2014, [18] who investigated that PEF processing of functional drink based on tender coconut water (*Cococus ucifera* L.) – nannari (*Hemidesmus indicus*) blended beverage. They found that PEF treatment also achieved a  $3.01 \pm 0.69$  log inactivation, similar to thermal pasteurization of native microflora. PEF treated tender coconut water-nannari blended beverage was stable up to 120 days under ambient storage condition (27-30°C).

HCIG treatment in this study can be explained by the electroporation effect on the cell structures, especially at the microbial cell membranes, which received the electric breakdown resulting in permeabilization to an osmotic process at the interior of the cell. The unequally accumulation of electrical charges on the two sides of cell membranes and the movements of the electrical charges

through the membrane leading to release of intracellular lipid, diffusion of solutes. Intensifying of the field strength and numbers of pulses in cell membranes can cause rupture of the membranes resulting in irreversible breakdown. Furthermore, the membrane conductivity increases after the pulse treatment resulting more membrane permeability leading to the cell death [20-25].

### 5 Conclusion

Application of nonthermal process revealed that HCIG reduced the microbial contamination under batch treatment at 5.17 kA, 9 pulses. This process was successful preserved the nutritional components for the coconut juice without significant physiochemical changes resulting the extension of shelf life of processed coconut juice. The HCIG model has a great potential to apply process for value added coconut juice especially for product export.

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