



# Exploration on the Effectiveness of Radio Frequency Treatment and Flash Pasteurisation on Enzyme and Microbial Activity for Coconut Water Preservation

Gnana Moorthy Eswaran<sup>1</sup>, Vidyalakshmi<sup>1\*</sup>, Tito Anand<sup>1</sup> and Vincent Hema<sup>1</sup>

<sup>1</sup>Indian Institute of Food Processing Technology, Thanjavur, India.

## Authors' contributions

*This work was carried out in collaboration among all authors. Author GME designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors Vidyalakshmi and TA managed the analyses of the study. Author VH managed the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

The aim of this research work is to explore the effect of flash pasteurisation (FP) and radio frequency (RF) treatment on enzyme activity, microbial activity and physicochemical properties of matured coconut water (MCW). The process conditions for FP were treatment temperature and time in combinations. The process conditions for RF treatment were different electrode position with constant current load 0.8 A. The results obtained from these treatments reveal that FP conditions had significant effect on inactivation of natural microbial flora, polyphenoloxidase (PPO) and peroxidase (POD) activity. From the results it is observed that FP at 90°C for 10 minutes is better for complete enzyme and microbial inactivation than RF treated MCW. The result is also evident that PPO has higher thermal stability than POD. From observed results the study also concludes that, FP is a better option for enzyme and microbial inactivation but RF treatment was found superior for retaining physicochemical attributes.

\*Corresponding author: E-mail: [rvidya@iifpt.edu.in](mailto:rvidya@iifpt.edu.in);

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## 1. INTRODUCTION

The coconut palm (*Cocosnucifera*) which belongs to the family *Arecaceae* is a monocotyledonous palm with no tap roots and the stems are unbranched. They grow well in tropics and are pan tropical. The coconut is botanically a drupe and not a nut. Globally India stands third in coconut production after Indonesia and Philippines. The matured coconut water (MCW) is a natural source of electrolytes, amino acids, vitamins, minerals and complex carbohydrates. Containing only 4 - 5% of carbohydrates, makes it suitable for sports drink. The calorific value of coconut water is 17.4 to 19 kcal per 100 g. Matured coconut water has the same level of electrolytic balance as we have in our blood [1].

Polyphenol oxidase (PPO) and Peroxidase (POD) which widely present in many fruits and vegetables are majorly linked to enzymatic colour changes with consequently affect the sensorial properties. Till date thermal treatment is most commonly used for enzyme inactivation in coconut water treatment. By considering the previous works [2,3] this study is based on the comparison of effect of thermal treatment (FP) and non-thermal (RF) treatment on enzyme activity and microbial activity in MCW. While thermal FP significantly reduces the enzyme activity and food borne diseases, it will also cause reduction in nutrient content as well due to its heating effect. RF is a non-thermal processing of food kept in between two electrodes in which high frequency directional electric field is generated thus imparts desired effects. Advantages of RF treatment over thermal treatment is, it has high penetration of electromagnetic power deep in to the samples thus causes uniform heating over sample geometry which in turn retains the nutritional attributes along with desirable effect on enzyme and microbial inactivation as describe in [4].

## 2. MATERIALS AND METHODS

### 2.1 Extraction and Preparation of CW

Matured coconuts between 10 to12 month age were purchased at the local market of Thanjavur, Tamil Nadu, India. The primary epicarp had been removed using dehusking equipment developed by Indian Institute of Food Processing

Technology, Thanjavur and washed with 100 ppm of active chlorine. The MCW was extracted manually using stainless steel punch of food grade quality for further processing and strained using muslin cloth to remove dust and coconut shreds.

### 2.2 Flash Pasteurization

Flash pasteurization (FP) of MCW was performed using hot water bath with various time and temperature combinations. Optimisation of pasteurization was done with 100 ml CW subjected to heat treatment with varying time and temperature combinations; 80°C, 85°C & 90°C for 2.5 min, 5 min, 7.5 & 10 min. After heat treatment the samples were immediately cooled in an ice-cold water bath before determination of pH, colour value, enzyme activity and microbial load.

### 2.3 Radio-Frequency Treatment

RF treatment was carried out using radio frequency sterilizer 10/4 insta assisted with an auxiliary hot air system. The RF power of 10 KW is applied to treat the coconut water which has been subjected to high voltage at frequency 40.68 MHz  $\pm$  0.05 MHz, the process parameters are varying electrode positions 200 mm, 240 mm, 280 mm, 320 mm and 360 mm with constant conveyor speed 10 m/hour which implies exposure time of 8.2 minutes and anode current of 0.8 A respectively. After treatment the samples were immediately cooled using ice-water bath before determination of enzyme activity and microbial load.

### 2.4 Enzyme Activity Evaluation

The POD activity was determined using pyrogallol as the substrate as performed in [2], with slight modifications. Each assay, 148  $\mu$ L distilled water, 20  $\mu$ L coconut water, 1 mL buffer solution of pH 6.0 (McIlvane solution) and 320  $\mu$ L of 5% (m/v) pyrogallol solution were mixed in a quartz cuvette. Subsequently, 160  $\mu$ L - 0.147 M H<sub>2</sub>O<sub>2</sub> solution was mixed, thus initiates the reaction. It was measured at 470nm for every 5s for 4 min using UV spectrophotometer for increase in absorbance of mixture solution.

The PPO activity was determined using catechol as the substrate as described in [5] with a slight

modification. In each assay, 2.3 mL buffer solution (McIlvane solution), 0.5 mL coconut water, 0.5 mL distilled water and 0.7 mL 4-methylcatechol 0.2M in buffer solution of pH 6.0 (McIlvane solution) were mixed in the quartz cuvette. It was measured at 420 nm for every 5s for 4 min using UV spectrophotometer for increase in absorbance of mixture solution.

Same procedure was performed for blank by substituting distilled water in place of coconut water. The PPO and POD activities were then expressed as per cent activity with reference to untreated control sample.

$$\% \text{Relative enzymatic activity} = (A/A_o) \times 100$$

Where,

A - Absorbance of enzyme activity before treatment

A<sub>o</sub> - Absorbance of enzyme activity after treatment

## 2.5 Microbial Activity

The total aerobic plate count of MCW was enumerated by serial dilution using sterilised buffered peptone water and plated on a plate count agar media. The results were observed at the end of incubation period for 24 hours at 37°C, the inactivation level of microbes was determined by evaluating the Log (CFU/ml) by counting number of colonies grown in media before and after the treatments.

## 2.6 Physicochemical Analysis

The physicochemical parameters of the MCW before and after treatments were analysed. The pH was measured using a pH meter. The Total

Soluble Solids (TSS) was determined directly using a digital refractometer at 20°C. Turbidity was determined using UV spectrophotometer at wavelength of 610 nm as described in [6]. Reducing sugar concentration was estimated by Di-nitro salicylic acid method and total sugar content was measured using acid hydrolysis as described in [7]. Protein content was estimated by Bradford's method. Total phenolic content was measured using Gallic acid method [8].

## 2.7 Statistical Analysis

All experiments were performed in triplicate. Results were reported as mean ± standard deviation values (n = 3). Factorial completely randomized design was followed for all the statistical analysis. Analysis of variance was performed to determine the significant effect of the independent variables on the response variables. The treatments and their interactions were compared at (p= .05) level using least square difference test, which was performed by SPSS V.22.

## 3. RESULTS AND DISCUSSION

### 3.1 Effect of FP on MCW

Fresh untreated coconut water analysed for physicochemical properties are summarized in Table 1. Then the samples were analysed for enzyme activity immediately after FP in which no significant difference was reported after treatment for 80°C but the per cent relative activity of PPO and POD gradually decreases with increase in temperature are shown in Fig. 1(a) and Fig. 1(b). Also there was significant decrease in microbial activity with increase in temperature as represented in Fig. 2. The result convinces the result obtained by [9,10].

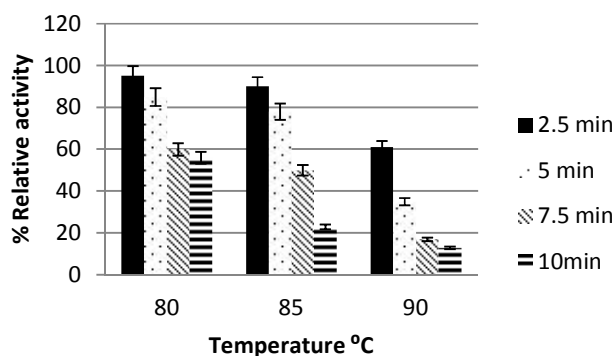


Fig. 1(a). Per cent relative activity of PPO for FPMCW

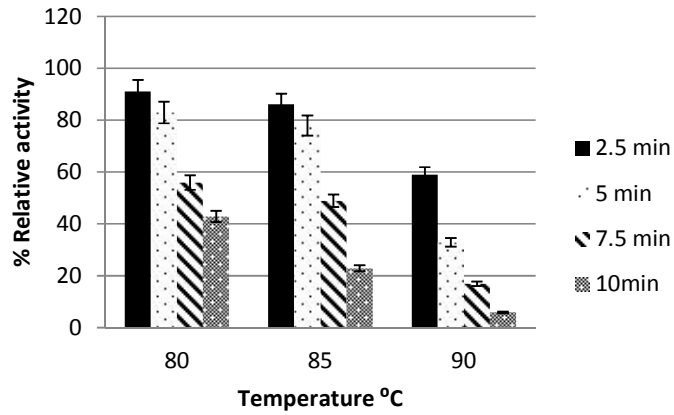


Fig. 1(b). Percent relative activity of POD for FPMCW

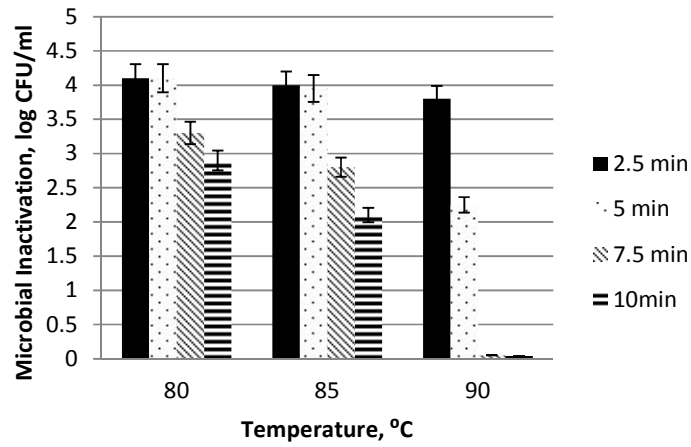


Fig. 2. Microbial inactivation kinetics for FPMCW with different time and temperature combination

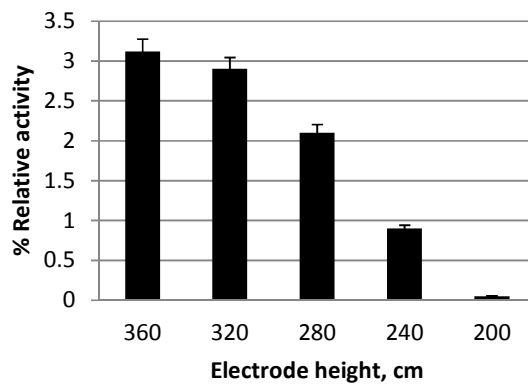


Fig. 3(a). Percent relative activity of PPO

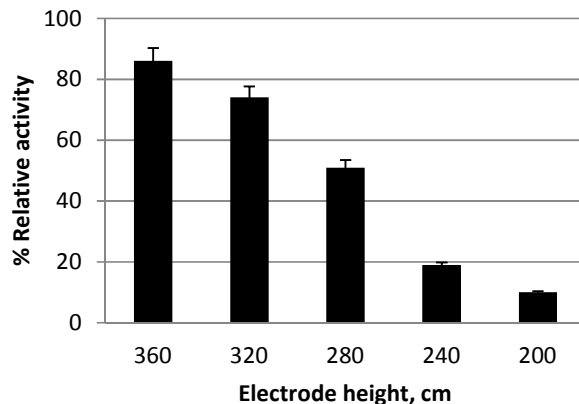


Fig. 3(b). Percent relative activity of POD

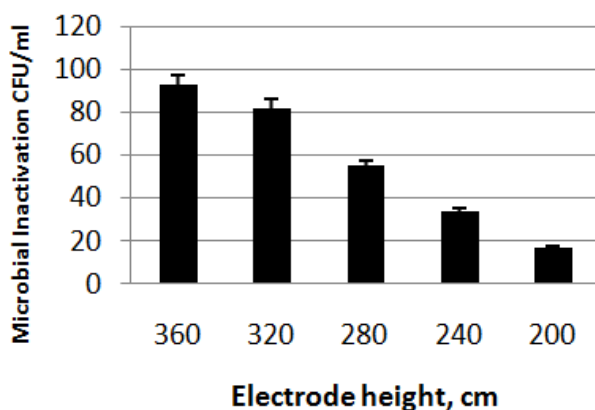


Fig. 4. Microbial inactivation kinetics for RF treated MCW with different electrode height

### 3.2 Effect of RF on MCW

The analysis of RF treated samples revealed a significant increase in per cent relative activity of PPO and POD initially, but it gradually decreased with decreasing electrode gap are represented in Figs. 3(a) and 3 (b) respectively. In Fig. 4 effect of RF on microbial inactivation, decreased microbial activity with decrease in electrode gap has been noted.

### 3.3 Optimisation of Treatments

From the observed results of FP and RF, 90°C for 10 minutes for thermal treatment and electrode height of 200 cm for RF treatment has been optimised as suitable treatment conditions respectively due to its effect on per cent relative activity of PPO and POD enzymes and microbial inactivation in MCW. The Analysis of variance

showed that temperature time combinations for FP treatment and electrode height for RF as well as their interaction were significant at ( $p = .05$ ). The extensive information on acceptability of RF treatment over FP treatment based on its inactivation mechanism of enzyme and native microbial flora along with perseverance of nutritional parameters has been determined by evaluating its quality parameters summarized in Table 1.

### 3.4 Discussion

The optimized FP-treatment ensued in a per cent relative PPO and POD activity of  $13.64 \pm 0.322\%$  and  $6.32 \pm 0.172\%$  in CW at 90°C for 10 min. RF was able to accomplish a similar per cent relative PPO and POD activity of  $17.69 \pm 0.118\%$  and  $10.08 \pm 0.215\%$  respectively for electrode gap of 200 cm for 8.2 min. The obtained results of

**Table 1. Physicochemical parameters, microbial log reduction and enzyme activity of treatment optimised CW**

Parameters	Untreated MCW	FP (90°C, 10min)	RF (200cm)
pH	5.07±0.114	5.07±0.148	5.06±0.712
TSS (° Brix)	4.93±0.017	5.27±0.091	5.16±0.119
Titration acidity (% citric acid)	0.059±0.009	0.061±0.008	0.058±0.006
Turbidity	3.043±0.321	3.657±0.246	3.172±0.041
Total Sugar (g/l)	36.73±1.412	35.18±1.064	36.53±1.616
Reducing sugar (g/l)	46.01±1.633	44.07±1.137	45.31±0.922
Protein Content (mg/l)	0.210±0.062	0.207±0.086	0.210±0.062
Total phenolic content (mg GAE / l)	24.47±1.751	19.47±1.491	23.75±1.237
Colour value (ΔE)	L 17.43±0.152 a* -0.43±0.063 b* -0.21±0.019	0.19±0.326	0.16±0.824
Microbial log reduction (CFU/ml)	1.4x10 <sup>4</sup>	0.04	0.04
Relative PPO activity (%)	100	13.64±0.322	17.69±0.118
Relative POD activity (%)	100	6.32±0.172	10.08±0.215

PPO and POD inactivation achieved were considered sufficient for good storage quality of MCW. Thus results of our study reveals that RF treatment positively affected the nutritive value of MCW in lesser time but with very less difference in enzyme and microbial inactivation than FP treatment. It is also evident in [11] where the effect of radio frequency on enzyme activity has been studied.

#### 4. CONCLUSION

Enzymes can present multiple states as response of processing. This complex behaviour depends on the system composition, enzyme conformation and processing properties. During radio frequency treatment, physical, mechanical or chemical factors directly affect the product and the three-dimensional structure of the enzyme. In this study, it was shown that the radio frequency treatment has the ability to decrease the enzyme activity depending upon the energy applied to the product, the current level and other properties related with the equipment design. This information can thus be exploited to the objectives, expanding the possible use of radio frequency industrially.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

#### REFERENCES

- Prades A, Dornier M, Diop N, Pain JP. Coconut water uses, composition and properties: A review. *Fruits*. 2012;67(2):87-107.
- Murasaki-Aliberti NDC, Da Silva RM, Gut JA, Tadini CC. Thermal inactivation of polyphenoloxidase and peroxidase in green coconut (*Cocos nucifera*) water. *International Journal of Food Science & Technology*. 2009;44(12):2662-2668.
- Tan TC, Cheng LH, Bhat R, Rusul G, Easa AM. Composition, physicochemical properties and thermal inactivation kinetics of polyphenol oxidase and peroxidase from coconut (*Cocos nucifera*) water obtained from immature, mature and overly-mature coconut. *Food Chemistry*. 2014;142:121-128.
- Marra F, Zhang L, Lyng JG. Radio frequency treatment of foods: Review of recent advances. *Journal of Food Engineering*. 2009;91(4):497-508.
- Augusto PED, Ibarz R, Garvín A, Ibarz A. Peroxidase (POD) and polyphenol oxidase (PPO) photo-inactivation in a coconut water model solution using ultraviolet (UV). *Food Res Int*. 2015;74:151-159. Available: <https://www.ncbi.nlm.nih.gov/pubmed/28411979> DOI: 10.1016/j.foodres.2015.04.046
- Rolle RS. Good practice for the small-scale production of bottled coconut water. *Food & Agriculture Org*. 2007;1.
- Jackson JC, Gordon A, Wizzard G, McCook K, Rolle R. Changes in chemical composition of coconut (*Cocos nucifera*) water during maturation of the fruit. *Journal of the Science of Food and Agriculture*. 2004;84(9):1049-1052.
- Delfiya DA, Thangavel K. Effect of ohmic heating on polyphenol oxidase activity,

- electrical and physicochemical properties of fresh tender coconut water. International Journal of Food Engineering. 2016;12(7): 691-700.
9. Ma Y, Xu L, Wang S, Xu Z, Liao X, Cheng Y. Comparison of the quality attributes of coconut waters by high-pressure processing and high-temperature short time during the refrigerated storage. Food Science & Nutrition; 2019.
  10. Sanganamoni S, Mahanti NK, Rao S. Modeling of polyphenol oxidase and peroxidase inactivation in coconut water during thermal treatment. IJCS. 2018;6(6): 1953-1958.
  11. Manzocco L, Anese M, Nicoli MC. Radiofrequency inactivation of oxidative food enzymes in model systems and apple derivatives. Food Research International. 2008;41(10):1044-1049.

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