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

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, polyphenol oxidase (PPO) and peroxidase (POD) activity. From the results it is observed that FP at 90 ºC 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.

Key words: FP, RF, Enzyme activity, microbial activity, physicochemical properties.

Introduction: The coconut palm (*Cocos nucifera*) 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 drinks. 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) are 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]

Materials and methods:

Extraction and Preparation of CW:

Matured coconuts between 10 to 12 months 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 100ppm 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.

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 100ml 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.

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 ± 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 10m/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.

Enzyme activity evaluation

The POD activity was determined using pyrogallol as the substrate as performed in [2], with slight modifications. Each assay, 148 µL water, 20 µL coconut water, 1 mL buffer solution of pH 6.0 (McIlvane solution) and 320 µL of 5% (m/v) pyrogallol solution were mixed in a quartz cuvette. Subsequently, 160 µL - 0.147 M H_2O_2 solution was mixed, thus initiates the reaction. It was measured at 470 nm 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 0.2 M 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.

%Relative enzymatic activity = (A/A_o) X 100

Where,

A - Absorbance of enzyme activity before treatment

A_o- Absorbance of enzyme activity after treatment

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** ℃, 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.

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].

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

Result and Discussion:

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 80ºC but the per cent relative activity of PPO and POD gradually decreases with increase in temperature are shown in Figure1(a) and Figure1(b). Also there was significant decrease in microbial activity with increase in temperature as represented in Figure 2. The result convinces the result obtained by [9] [10].

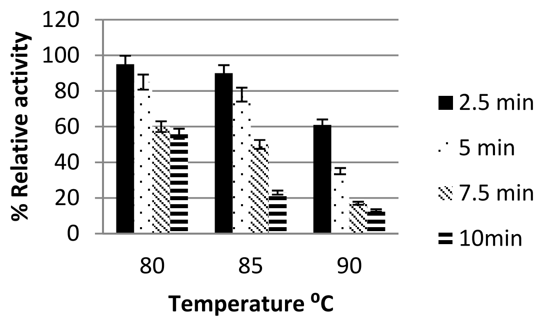

Figure 1(a). Per cent Relative activity of PPO for FP MCW

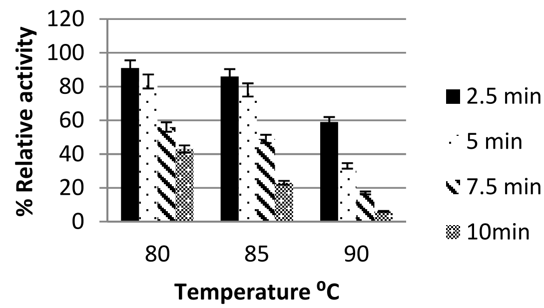

Figure 1(b). Per cent Relative activity of POD for FP MCW

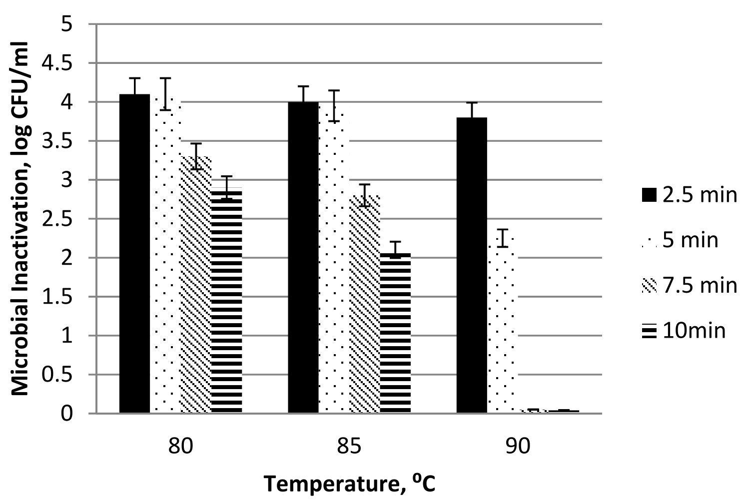

Figure 2. Microbial inactivation kinetics for FP MCW with different time &temperature combination

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 Figure 3 (a) &Figure 3 (b) respectively. In Figure 4 effect of RF on microbial inactivation, decreased microbial activity with decrease in electrode gap has been noted.

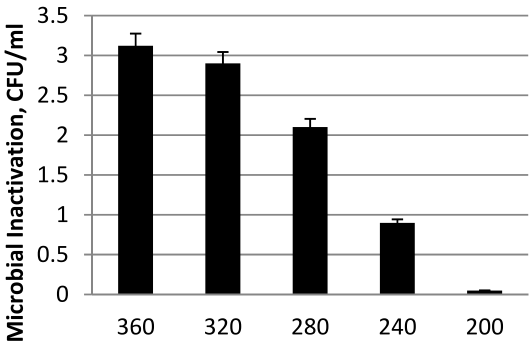

Figure 3(a).Per cent Relative activity of PPO for

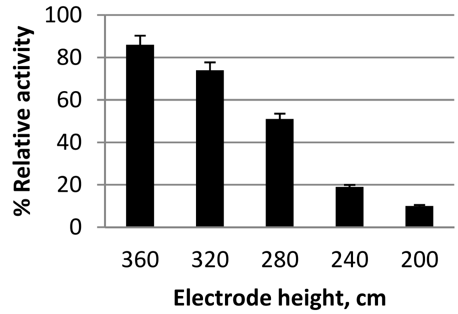

Figure 3(b).Per cent Relative activity of POD

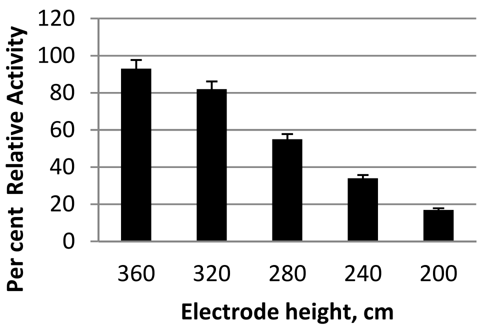

Figure 4. Microbial inactivation kinetics for RF treated MCW with different electrode height

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.

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
Titrable 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□	0.04	0.04
Relative PPO activity (%) Relative POD activity (%)	100 100	13.64±0.322 6.32±0.172	17.69±0.118 10.08±0.215

Conclusion:

The optimized FP-treatment ensued in a per cent relative PPO and POD activity of 13.64 ± 0.322 and 6.32 ± 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 ± 0.118 and 10.08 ± 0.215 respectively for electrode gap of 200 cm for 8.2 min. The obtained results of 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.

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