EFFECT OF THERMAL AND NON-THERMAL PROCESSING TECHNIQUES ON PHYSICO-CHEMICAL, MICROBIOLOGICAL AND SENSORY PROPERTIES OF READY TO SERVE HEALTH BEVERAGE

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ABSTRACT

A study was designed to test the effect of non-thermal processing techniques vs thermal processing in the production of a ready to serve health beverage by blending curry leaf (*Murraya koenigii*), star fruit (*Averrhoa carambola*), watermelon (*Citrullus lanatus*), and Aloe vera (*Aloe barbadensis* Miller) juices. Juices were pasteurized, processed with ultrasound with varied ultrasonic power and time combinations and micro-filtered. Microbiological, organoleptic, physico-chemical parameters, proximate composition and antioxidant activity of processed juices were evaluated. Results of the microbiological analysis of the ultrasonically processed and micro-filtrated juice indicated the effectiveness to obtain microbiologically safe juice compared to thermal processing. Antioxidant capacity increase with ultrasonic processing and reduces with the thermal processing and microfiltration

***Key words*:** Health beverage, Thermal and non-thermal technique, Antioxidant activities

1. INTRODUCTION

Current consumer trend for healthy drinking has provided a quite stable platform for innovative juice market [1, 2]. There is a distinct correlation in the use of medicinal plant beverages with the age thus, the tendency is high among middle aged, being more health conscious. Epidemiologic studies shows that fruit, vegetable and herbs consumption has been closely associated with decreased incident and mortality from a diversity of negative health outcomes including obesity, hypertension, cardiovascular diseases, cancer, and stroke. [3, 4]. These are due to phytochemicals, and have been reported to have extensive health benefits including antioxidant, anti-inflammatory, lipid-lowering and beneficial effects on endothelial function [5]. In the preservation of juices, pasteurization (PZ) ensures prevention of microbiological deterioration by thermal destruction, elimination of Oxygen and prevention of enzymatic action. Pasteurization causes some effects on organoleptic and/or nutritional properties of juices. Although some of these changes may be desirable, the rather harsh temperature for an extended time period would trigger chemical reactions and loss of nutrients, freshness and sensory characteristics [6]. Non-thermal juice preservation techniques like microfiltration (MF) and ultrasonic (US) processing is worthy to investigate to eliminate some of these ill effects. Ultrasound comprises vibrations similar to sound waves between 18 kHz -500 MHz which, in a biological media, produce cycles of compression and expansion and subsequently the phenomenon of cavitation [7]. High-intensity cavitation punctures the cell walls of the microorganisms and releases the cell content [8, 9]. Microfiltration (MF) has a great attention for clarifying and stabilizing purpose of raw fruit juices [10, 11

2. METHODOLOGY

2.1. Procedure for Analysis

Fresh curry leaves (*Murraya koenigii*), star fruits (*Averrhoa carambola*),watermelons **(***Citrullus lanatus***)** and Aloe vera (*Aloe barbadensis* Miller) were purchased from the Narahenpita market and were cleaned by washing three times with potable water, with 200 ppm chlorine solution, and again three times with potable water. Curry leaves were blended with potable water and filtered through, 1.18mm sieve and 300μm sieve.

The juice was heated at 80˚C for 5 minutes, then cooled to room temperature and filtered through a clean muslin cloth. Star-fruits juice was extracted and filtered through a clean muslin cloth. The sugar melons were cut in to pieces and de-seeded. The pieces were blended and the obtained juice was filtered through a clean muslin cloth. Bottom part of the Aloe vera leaves was cut and marked lines in the leave from top to bottom with a sharp knife. Leaves were placed erect inside a bucket overnight to remove bitterness. Peeled off the leaves and gel was removed by cleaned sharp knife. Gel parts were cut into tiny pieces and washed thoroughly with portable water and get the pieces alone. The gel pieces were mixed with 3% citric acid and left 10 minutes and washed with potable water. The pieces were blended and filtered through a clean muslin cloth. The extracted curry leaf, star fruit, sugar melon and Aloe vera juices were mixed according to the formula developed. Citric acid was added to adjust the final pH to 3.5 and food grade sugar was added to adjust the final brix value of 9˚Brx. The blended juice weight was recorded and divided into three samples. An ultrasonic juice processor (SJIA–1500 W, Ningbo Yinzhou Sjia Lab Equipment Co., Ltd.) with a 15 mm probe was used for sonication. Based on the results of preliminary tests, range of increasing treatment of ultrasound wave's power and time were applied to the juice sample of unprocessed blended juice (Table 1). Samples were processed at a constant frequency of 20 kHz, temperature which was maintained under 40˚C and pulse duration (S) of 1.5 on and 2.5 off.

In the microfiltration experiment, the polyvinylidene fluoride membrane (0.3µm) was inserted to the experimental membrane filtration unit (Figure 1) and the unit was cleaned twice by running 200 ppm chlorox solution and potable water. The prepared second juice sample was micro filtered (0.5 MPa) The feed was swapped from time to time when its temperature reached 40˚C and temperature of the feed brought to ambient temperature (± 30^{o}C). Permeate was filled in to sterilized 200 ml polyethylene terephthalate bottles (PET**)** bottles and capped. The samples for microbiological analysis were collected in to sterilized sachets. Rest of the blended and unprocessed juice sample was heated until the temperature reached 80˚C. Sodium metabisulphite was added (SLS 729-2010) and mixed well. The heated juice was then filled in to the PET bottles and capped. The sealed bottles were pasteurized for 20 minutes by immersing in a boiling water bath (80 ^{o}C). The bottles were allowed to cool, labeled and stored in the refrigerator (4˚C). Samples obtained from ultrasonic processing, microfiltration and pasteurization were tested for microbial safety (SLS 516: **yea**r) by subjecting them to aerobic plate count, yeasts and moulds count, and total coliform/ E. coli count test. Total soluble solids (TSS), acidity, reducing sugar, pH value and proximate composition analysis were determined. The Total Phenolic Content (TPC) of juice samples was determined by the Folin-Ciocalteu reagent using 96-well micrpolates (n=3) [12]. DPPH radical scavenging assay was performed in 96-well micro-plates (n=3) [13]. The ABTS+ radical scavenging activity was determined in 96-well micro-plates (n=3) [14]. Appearance, color, odor, taste, after taste, and overall acceptability of the pasteurized, micro filtrated and ultrasonically processed juice samples were evaluated by using 15 trained sensory panelists based on the 9 point hedonic scale

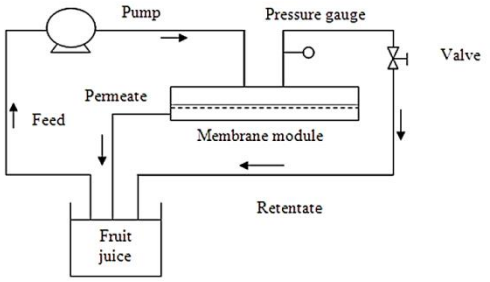

Figure 1. Schematic diagram of microfiltration unit

The statistical analysis of data were carried out using ANOVA by the statistical software SAS® and by Kruskal-Wallis method using the software MINITAB® 14.

3. RESULTS

To comply with ready serve drinks safety standard the APC should be below 50 per ml and there should not be Yeast and Mould and E-coli in the sample. Results showed (Table 1) that the all US treatment combinations and pasteurized samples are complying with the standard. Thus, results indicated that complete microbial destruction have not been achieved by the ultrasonic treatments. Due to the excess APC counts untreated sample did not comply with the standard. Microbiological assessment of the pasteurized and micro-filtered juices (Table 2) indicated that these samples are complying with the standard compared to untreated samples.

Results showed that the ultrasonic and microfiltration techniques can be used as a non-

Table1. Results of microbiological analysis of ultrasonically processed juice (per ml)

US Power (W)	Time (Min.)	APC	Yeast & mould	Total coliform & *E. coli*
200	14	4	6	Nil
600	10	4	Nil	Nil
800	8	2	Nil	Nil
1000	6	4	Nil	Nil
1200	4	17	Nil	Nil
1400	2	4	Nil	Nil
Control		75	6	Nil
Pasteurized		Nil	Nil	Nil
Standard		<50	Nil	Nil

Table 2 Results of microbiological analysis of micro filtrated and thermally processed samples (per ml)

Colonies	Control	MF	PZ
APC	60	Nil	4
Yeast &Mould	Nil	11	Nil
Total coliform/ *E. coli*	Nil	Nil	Nil

thermal technique to obtain microbiologically safe sample. Results of the total polyphenolic content (TPC) showed (Table 3) that TPC (mg gallic acid equivalents/200ml) of juice has increased after pasteurization and decreased after microfiltration.

Table 3.Total polyphenolic content and DPPH radical scavenging activity of thermally processed and micro-filtered juice samples

Sample	TPC	DPPH	$ABTS^+$
Control	256.9 $\pm$ 0.7^b	240.8 $\pm$ 7.1^a	975.3 $\pm$ 6.2^a
MF	233.3 $\pm$ 9.6^c	186.5 $\pm$0.7^b	715.6 $\pm$ 42.4^c
PZ	279.5 $\pm$ 0.0^a	83.6 $\pm$ 16.0^c	790.4 $\pm$ 23.4^b

Data represented as mean $\pm$ SE (n=3). Mean values in a column superscripted by different letters are significantly different at $p<0.05$

DPPH Radical scavenging activity (mg of trolox equivalents / 200ml) has decreased in both thermal processing and microfiltration processes. $ABTS^+$ radical scavenging activity (mg of trolox equivalents / 200ml) has decreased in pasteurized and micro -filtrated samples. Loss of antioxidant capacity in microfiltration is not due to destruction and that is because of the filtration effect. Results of ultrasonic processing (Table 4) indicated that ultrasound treatment affects the TPC value to significantly increase than the increase of TPC by pasteurization. Radical scavenging activity showed that significant increment compared to significant decrement by pasteurization. Results indicated that there is no significant difference ($p > 0.05$) between overall sensory acceptability of microfiltrated and pasteurized juices (Table 5) thus, between overall acceptability of pasteurized and ultrasonically processed juices (Table 6).

Table 4 Total polyphenolic content, DPPH and ABTS+ radical scavenging activities of untreated pasteurized, and ultrasonically processed juice

Sample	TPC	DPPH	$ABTS^+$
Control	256.93 $\pm$ 0.75^e	400.54 $\pm$ 22.12^{ab}	1227.04 $\pm$ 13.69^{cd}
Pasteurized	279.59 $\pm$ 0.00^d	240.81 $\pm$ 7.16^d	975.32 $\pm$ 6.26^e
200 W 14 min	334.07 $\pm$ 14.31^c	311.07 $\pm$ 4.95^c	1247.74 $\pm$ 21.75^{bc}
600 W 10 min	363.70 $\pm$ 8.73^b	407.37 $\pm$ 9.44^a	1270.37 $\pm$ 3.40^b
800 W 8 min	359.26 $\pm$ 6.58^b	352.98 $\pm$ 29.78^{bc}	1245.40 $\pm$ 13.31^{bc}
1000 W 6 min	356.78 $\pm$ 2.64^b	354.28 $\pm$ 13.21^{bc}	1196.21 $\pm$ 11.37^d
1200 W 4 min	377.78 $\pm$ 5.13^{ab}	370.71 $\pm$ 5.05^{ab}	1277.39 $\pm$ 2.07^b
1400 W 2 min	387.41 $\pm$ 9.10^a	371.51 $\pm$ 0.40^{ab}	1315.62 $\pm$ 8.22^a

Data represented as mean $\pm$ SE (n=3) Means values in a column superscripted by different letters are significantly different at $p<0.05$

Table 5 Results of sensory evaluation of pasteurized and micro-filtered (MF) juices

Parameter	PZ	MF
Appearance	5.0 $\pm$ 1.4	6.8 $\pm$ 1.3
Colour	5.6 $\pm$ 0.8	6.6 $\pm$ 1.1
Ordor	6.2 $\pm$ 1.6	5.6 $\pm$ 5.6
Taste	5.4 $\pm$ 1.1	5.4 $\pm$ 2.0
After taste	5.4 $\pm$ 1.1	5.6 $\pm$ 1.9
Overall	5.4 $\pm$ 1.1	5.8 $\pm$ 1.9

Present study focused to test the affectivity of non-thermal techniques on microbial safety and chemical properties of selected juices. Thus,

there is a possibility to improve sensory acceptability thereby overall juice qualities.

Table 6 Results of sensory evaluation of pasteurized ultrasonically processed juice

parameter	PZ	UP
Appearance	5.4 ± 1.27	6.4 ± 0.98
Colour	5.9 ± 0.90	6.3 ± 0.95
Taste	6.4 ± 1.27	6.1 ± 0.69
After taste	5.9 ± 1.68	5.7 ± 1.12
Overall	6.1 ± 0.84	6.3 ± 1.11

Data represented as mean ± SD (n=5).

Efficacy of ultrasound treatment with heat or pressure or both heat and pressure [15] is worthy to further study to improve the juice quality.

4. CONCLUSION

Microfiltration and ultrasonic processing can be used as an effective non-thermal technique to process ready to serve beverage. Antioxidant capacity is reduced with thermal processing and microfiltration. Ultrasonic processing retains the highest antioxidants activity compared to thermal processing and microfiltration. Further studies are being carried out to confirm the best ultrasonic treatment combination and improving of overall juice quality by non-thermal techniques.

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