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Effects of Non-Thermal Processing Methods on Physicochemical, Bioactive, and Microbiological Properties of Fresh Pineapple (*Ananas comosus* L. Merr.) Juice

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HIGHLIGHTS

- Ultra-sonication improved the total antioxidant capacity in pineapple juice.
- Mild pasteurization and ultra-sonication demonstrated greater microbial inactivation.
- Non-thermal technologies yielded very little change in the color attributes.

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Acronyms and abbreviations
CFU=Colony Forming Unit
DPPH=2,2-Diphenyl 2-Picrylhydrazyl
GAE=Gallic Acid Equivalents
TAC=Total Anthocyanin Content
TBC=Total Bacterial Count
TPC=Total Phenolic Content
TSS=Total Soluble Solids

ABSTRACT

Background: Pineapple juice processing is an art of preservation, and the processing technologies play important role in pineapple juice quality. Therefore, this study aimed to explore the potential impacts of non-thermal processing methods on the physicochemical, bioactive, and microbiological properties of fresh pineapple juice.

Methods: Extracted juices were subjected to several non-thermal processes including microwave processing, vacuum evaporation, mild pasteurization, pulsed electric field, and ultra-sonication. Physicochemical properties including Total Soluble Solids (TSS), pH, titratable acidity, and color; Total Phenolic Content (TPC); Total Anthocyanin Content (TAC); antioxidant capacity; and microbiological properties were evaluated. Data were statistically analyzed by Minitab statistical software (version: 18.1).

Results: TSS, pH, acidity (%) of processed juices ranged from 11.03-12.03, 4.07-4.27, and 0.42-0.49, respectively. In terms of color properties both ultra-sonication and microwave processing showed the highest values of L*(luminosity), a* (redness), and b* (yellow). The highest TPC was reported in ultra-sonication treatment 11.996±0.002 mg Gallic Acid Equivalents (GAE)/100 ml. The TAC varied from 0.179-0.235 mg Total Anthocyanin (TA)/100 ml, where ultra-sonication and mild pasteurization treatment yielded the highest and lowest contents, respectively.

Conclusion: Perfect phenolic content, antioxidant capacity, retention of anthocyanin content, and attractive color in pineapple juices when treated with non-thermal techniques.

Introduction

Pineapple (*Ananas comosus* L. Merr.) is one of the major commercial and popular fruits in Bangladesh. This fruit can be consumed either raw, fresh, or processed. However, fresh pineapple juice is extremely popular due

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to its flavor, pleasant aroma, and numerous functional properties (Khalid et al., 2016). In addition, pineapple is an excellent source of natural fiber and various antioxidant compounds such as polyphenols, carotenes, and vitamins (Rizzo and Muratore, 2009). These phytocompounds play an important role in overall well-being of humans. The high antioxidant activity and vitamin C content of pineapple juice links to numerous health benefits such as wound healing and also as an anti-inflammatory agent in the healing of soft tissue injury; treatment of arthritis; hematoma; and necrotic tissue (Saxena and Panjwani, 2014).

However, the climate and the soil of many parts of Bangladesh are suitable for pineapple production. The diverse uses of pineapple result in the development of numerous fruit processing industries in Bangladesh. However, long-term preservation of fruit juices becomes a growing concern, as microorganisms, spoilage enzymes, and undesirable chemical reactions which can impair the quality and sensory attributes (Aneja et al., 2014). Moreover, ripe pineapple fruits are typically spoiled by a variety of fungi species and the juices is found to be spoiled by improper handling soon after harvesting (Auta et al., 2017). Consumers today are looking for food products that are more nutritious and safer to consume (Walkling-Ribeiro et al., 2009). Previously, it was reported that some chemical preservatives, such as sorbate and benzoate, were used to extend the shelf life of beverages (Nwachukwu and Ezeigbo, 2013). However, the use of those chemical preservatives in food tends to have adverse effects on the health of consumers (Adesokan et al., 2010). Furthermore, despite showing negative impact on the organoleptic quality and bioactive properties, conventional thermal processing and high temperature treatment remain the most widely used technology for the shelf-life extension and preservation of fruit juices (Rawson et al., 2011).

To address these issues, researchers came up with several non-thermal processing methods which are proven to decrease microbial loads and spoilage enzymes without sacrificing the flavor, color, and bioactive compounds (Charles-Rodríguez et al., 2007). Some of the non-thermal processing methods including ultra-sonication, pulsed electric field, microwave, as well as irradiation processing are proposed during the last couple of decades (Bhat et al., 2011; Devlieghere et al., 2004; Tiwari et al., 2009). These non-thermal technologies receive considerable attention for a variety of applications, including fruit juice processing to preserve sensorial quality and nutrient values (Zhang et al., 2019). Despite the availability of numerous reports on the effects of non-thermal treatments combined with heat pasteurization on the nutrients and antioxidant activities, there is a lack of information on pineapple juice. Therefore, the study aimed to evaluate the comparative effects of microwave processing, vacuum evaporation, mild pasteurization, pulsed electric field, and ultra-sonication on the physicochemical, bioactive, and microbiological properties of pineapple juice.

Materials and methods

Raw materials and juice extraction

Raw and fresh pineapples were purchased from local market in Chattogram, Bangladesh. Pineapple fruits and raw materials were washed and rinsed thoroughly with potable water. The fruits were peeled and cut into small pieces with a sterile knife and further homogenized in a sterile mixer grinder (Panasonic MX-AC300, India). The extracted juice was then filtered by passing through a sterile muslin cloth and the resulting juice was vortex mixed in 250 ml polypropylene bottles and divided into 6 different parts as control sample and working samples for non-thermal processing (Akinosun, 2010).

Study design

The bottles were kept at -20 °C until treatment, at which point they were allowed to thaw at room temperature. The freshly extracted juice, however, without any treatment, was chosen as the control. The working samples were further subjected to following non-thermal treatments. Each treatment was performed in triplicate.

Microwave processing

Microwave oven (Panasonic NN-CD9978, Japan) at 2,450 MHz was used for the treatment of pineapple juice. Each 50 ml of fresh pineapple juice sample in a 250 ml beaker was placed in microwave oven for 20 s. As soon as there was a sign of boiling, the sample was removed with gloves. It was then cooled until the temperature dropped to 25 °C and stored for further analysis (Khalil, 2019).

Vacuum evaporation

Fifty ml of fresh pineapple juice was placed in round bottom flask of rotary vacuum evaporator (Heidolph Hei-VAP series, Germany) with built-in water bath and vacuum pump (Buchi Vacuum Controller V-850, India). During the evaporation process, the sample was heated by immersing it in water bath (55 ℃) and warmed by a 1,050 W electrical power heater. Samples were cooled immediately after the treatment and stored at room temperature (25 °C) for further analysis (Sabanci et al., 2019).

Mild pasteurization

Polycarbonate basin constructed water bath (Veekay Testlab, India) was used for the mild pasteurization of pineapple juice. Fifty ml of clarified pineapple juice was transferred into a clean sterile closed glass jar and pasteurized at 65 °C for 20 s. After the treatment, samples were cooled immediately and stored at room temperature (25 °C) for further analysis (Lagnika et al., 2017).

Pulsed electric field treatment

A laboratory scale pulsed electric field device was designed and constructed which is consisted of power supply, pulse generation, and computational control, high voltage level shifter, and a reactor unit as described by Yousuf et al. (2020). A simple cuvette worked as reactor where 2 copper electrodes were placed at a distance of 2 mm and the sample volume was 1 ml. Fifty ml of fresh pineapple juice sample was poured in batch between the electrodes of pulsed electric field device. The processing conditions included electric field strength at 5 kV/cm and total treatment time in 20 min. After heating juice samples were cooled to room temperature (25 °C) and stored for further analysis (Yousuf et al., 2020).

Ultra-sonication

Fifty ml of pineapple juice sample was taken in a 250 ml Erlenmeyer flask. Subsequently, the sonication temperature was adjusted to 25±5 °C while placing the flask in the thermostatic sonicated bath (Power Sonic 520, Korea). The processing conditions included the frequency of 40 kHz with the constant power of 500 W for 15 min. After the treatment, juice samples were immediately cooled and stored at room temperature (25 °C) for further analysis (Ruiz-De Anda et al., 2019).

Physicochemical properties

-Total Soluble Solids (TSS; °Brix), pH, and titratable acidity

The °Brix of juice sample was measured at 25±1 °C using a hand refractometer (Hanna Instrument, Italy). All the measurements were carried out in triplicates and the prism of refractometer was washed with distilled water after each analysis. The pH of pineapple juice sample was measured using a digital pH meter (Hanna Instrument, Italy). Ten ml of sample was placed in a beaker and stirred continuously with a magnetic stirrer and the pH was measured at 25±1 °C, and the pH meter was calibrated with commercial buffer solutions of pH 7.0 and 4.0 prior to each measurement. Titratable acidity was determined according to standard AOAC method (AOAC, 1999). Ten ml of juice sample was placed into a 250 ml beaker and 90 ml distilled water and 3 drops of phenolphthalein indicator was also added. The mixture was titrated against standardized solution of 0.1 N NaOH to the end point. The volume of NaOH was converted to amount of malic acid (g) per 100 ml of juice and titratable acidity was then calculated by using following equation:

$$Titratable\ acidity\ (\%) = \frac{\text{ml NaOH} \times \text{Normality of NaOH} \times \text{Acid factor} \times 100}{\text{Sample Volume in ml}}$$

-Color attributes

Color properties were measured according to the method described by Wu and Sun (2013) using Hunterlab ColourFlex spectrophotometer (Management Company, USA) as L*, a*, and b* values, where L* measures luminosity on a scale from 0 to 100 (from black to white), a* indicates (+) red or (-) green; and b* indicates (+) yellow or (-) blue. The total color difference (ΔE), chroma (C), hue angle (h) was also calculated using the following equations:

$$\Delta E = \sqrt{(L - L0)^2 + (a - a0)^2 + (b - b0)^2}$$

$$C = \sqrt{a^2 + b^2}$$

$$h = tan - 1\ \frac{(b)}{(a)}$$

Here, $L0$, $a0$, and $b0$ represent the values of control juice.

Bioactive properties

-Determination of Total Phenolic Content (TPC)

TPC of pineapple juice samples were determined according to Folin-Ciocalteu method described by Alothman et al. (2009) with slight modifications. Three tenths ml samples were pipetted into a cuvette containing 1.5 ml diluted FC reagent (1:10). The solutions were mixed thoroughly and left for 3 min at room temperature. Then, 1.5 ml of sodium carbonate (7.5%) solution was added and again incubated at room temperature for 60 min. The absorbance was taken at the wavelength of 765 nm using a UV visible spectrophotometer (UV-2,600, Shimadzu Corporation, USA) and ethanol was used as the blank. For quantification of TPC, a gallic acid standard curve was plotted with different standard solutions of gallic acid i.e., 0.02, 0.04, 0.06, 0.08, and 0.10 mg/ml. The absorbance of the juice samples were compared against the standard curve of gallic acid. TPC was calculated and expressed as mg of Gallic Acid Equivalents (GAE) per 100 ml of samples (mg GAE/100 ml).

-Determination of Total Anthocyanin Content (TAC)

TAC of the pineapple juice samples were determined colorimetrically by following method described by Selim et al. (2008) with slight modifications. Three ml of the sample was pipetted into a cuvette and the intensity of color was measured at the wavelength of 520 nm using UV visible spectrophotometer (UV-2,600, Shimadzu Corporation, USA). Ethanol was used as the blank. TAC was calculated and expressed as mg per 100 ml (mg/100 ml) using the following equation:

$$\text{TAC}=\frac{Absorbance\ of\ sample \times DF \times 100}{m \times E}$$

Here, DF stands for Dilution Factor; m represents the weight of sample; E refers to extinction coefficient (55.9).

-Determination of antioxidant capacity

Antioxidant capacity of the pineapple juice samples was determined using 2,2-Diphenyl 2-Picrylhydrazyl (DPPH) assay as the method described by Chan et al. (2007) with slight modifications. Six mg of DPPH was dissolved in 100 ml methanol to prepare methanolic DPPH solution. An aliquot (0.5 ml) of sample was added to 2.5 ml of methanolic DPPH solution. The mixture was gently shaken and left for 30 min in dark at room temperature. The absorbance was taken at the wavelength of 517 nm using UV visible spectrophotometer. Control was prepared by mixing 1 ml of methanol with 2 ml of DPPH solution, while methanol was used as the blank. The scavenging activity was measured as the decrease in absorbance of the samples in comparison with the DPPH standard solution. Antioxidant capacity based on the DPPH free radical scavenging ability of juice samples was calculated using the following equation:

$$\text{Inhibition (\%)}=(1-\frac{Absorbance\ of\ sample}{Absorbance\ of\ control})\times 100$$

Trolox was used as standard and for each dilution of juice; antioxidant capacity was calculated on the basis of the trolox calibration curve and expressed in μmol of Trolox Equivalent (TE) per ml of fruit juice.

-Determination of microbiological properties

Total Bacterial Count (TBC) of the juice samples was determined according to the method described by Santhirasegaram et al. (2013). Serial dilution bottles were filled with 0.1% peptone water and further autoclaved. Juice samples were appropriately diluted (up to 10^5 times). These dilutions were then poured into sterile petri dishes. Molten agar (15 ml) was added immediately to each petri dish. Then, the petri dishes were stored at 37 °C for 48 h in an incubator (GSP-9,080 MBE, Shanghai, China). The TBC was calculated as Colony Forming Units per ml (CFU/ml) of juice according to the following equation:

$$\text{CFU/ml}=\frac{Number\ of\ colonies \times Dilution\ factor}{Volume\ of\ culture\ plate}$$

Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) using Minitab Statistical Software (Version 18.1) followed by Fisher’s LSD test to determine statistical differences among the processing methods to test the level of significance ($p<0.05$). Obtained data were presented as Mean Value±Standard Deviation (SD).

Results

Physicochemical properties

The effects of non-thermal processing methods on the physicochemical properties of control and processed pineapple juices are shown in Table 1. The TSS of the control and processed juice samples varied from 11.03 to 12.06 (°Brix), whereas the TSS of the pulsed electric field and ultra-sonication treatments was significantly lower ($p<0.05$). Furthermore, the pH of the treated samples (vacuum evaporation, pulsed electric field, and ultra-sonication) ranged from 4.07 to 4.27, indicating statistically significant differences ($p<0.05$) between these values and the control. However, the pH of the control, microwave processed, and mild pasteurized juices showed no significant differences ($p>0.05$). In terms of titratable acidity, mild pasteurized juice (0.49%) had significantly higher acidity than control juice (0.46%), while ultra-sonication produced the least acidity (0.42%).

Color attributes

Table 2 shows the color attributes of the control and treated pineapple juices, including their chromatic coordinates (L*, a*, b*), color difference (ΔE), chroma (C), and hue angles (h). The L*, a*, and b* values of the control pineapple juice sample were 43.99, 5.54, and 13.70, respectively, and regardless of a* value in vacuum evaporation, all processing methods caused significant changes in these values ($p<0.05$). L*, a*, and b* values ranged from 37.89 to 48.07, 5.57 to 7.12, and 11.09 to 16.84, respectively, regardless of processing method. Accordingly, the color difference (E), chroma (C), and hue angles (h) of the treated pineapple juice ranged from 4.13 to 6.19, 14.23 to 18.28, and 63.15 to 67.08, indicating significant differences ($p<0.05$) between these values and the control.

Bioactive properties

The effects of non-thermal processing methods on the bioactive compounds of control and processed pineapple juices are shown in Table 3. TPC levels in pineapple juice ranged from 11.14 to 11.09 mg GAE/100 ml, with significant differences (p<0.05) between the control and processed juices. As a result, ultra-sonication and pulsed electric field treatment had the highest and lowest TPC, respectively. Furthermore, the TAC of the treated samples (mild pasteurization, vacuum evaporation, and ultra-sonication) ranged from 0.17 to 0.23 (mg TA/100 ml), indicating significant differences (p<0.05). TAC of the control, microwave processed, and pulsed electric field treatments, on the other hand, showed no significant differences (p>0.05). However, the antioxidant capacity of pineapple juices was significantly (p<0.05) improved by all the processing methods.

Microbiological properties

Table 4 shows the effects of non-thermal processing methods on the total bacterial count of control and processed pineapple juices. Obtained result demonstrated the highest bacterial count in control juice (9.50×10^4 CFU/ml). However, TBC of the treated samples (mild pasteurization, ultra-sonication, microwave processing, pulsed electric field, and vacuum evaporation) varied from 2.80 to 6.30 (10^4 CFU/ml) suggesting significant differences (p<0.05) between these values and control group.

Table 1: Physicochemical properties of control and treated pineapple juices

Treatments	TSS (°Brix)	pH	Titratable acidity (%)
Control	12.06±0.21 ^{a}	4.14±0.02 ^{c}	0.46±0.01 ^{b}
Microwave	12.00±0.20 ^{a}	4.16±0.01^c	0.43±0.01 ^{d}
Vacuum evaporation	12.03±0.35 ^{a}	4.27±0.02 ^{a}	0.45±0.01 ^{c}
Mild pasteurization	11.97±0.15 ^{a}	4.13±0.02 ^{c}	0.49±0.01 ^{a}
Pulsed electric field	11.03±0.15 ^{b}	4.22±0.02 ^{b}	0.46±0.02 ^{b}
Ultra-sonication	11.50±0.30 ^{b}	4.07±0.02 ^{d}	0.42±0.03 ^{e}

Data in same column with different letters are significantly different (p<0.05).
TSS=Total Soluble Solids

Table 2: Color properties of control and treated pineapple juices

Treatments	L*	a*	b*	ΔE	C	h
Control	43.99±0.76 ^{b}	+5.54±0.03 ^{e}	+13.70±0.15 ^{c}		14.78±0.02 ^{d}	67.98±0.02 ^{a}
Microwave	40.92±0.71 ^{c}	+7.12±0.03 ^{a}	+16.84±0.02 ^{a}	4.67±0.15 ^{c}	18.28±0.02 ^{a}	67.08±0.01 ^{b}
Vacuum evaporation	39.16±0.59 ^{d}	+5.57±0.05 ^{e}	+11.09±0.03 ^{f}	5.49±0.02 ^{b}	12.41±0.01 ^{f}	63.33±0.03 ^{e}
Mild Pasteurization	40.07±0.62 ^{cd}	+6.82±0.03 ^{b}	+13.47±0.03 ^{d}	4.13±0.02 ^{e}	15.09±0.02 ^{c}	63.15±0.03 ^{f}
Pulse electric field	37.89±0.69 ^{e}	+6.19±0.04 ^{d}	+12.82±0.02 ^{e}	6.19±0.01 ^{a}	14.23±0.02 ^{e}	64.23±0.02 ^{d}
Ultra-sonication	48.07±0.20 ^{a}	+6.45±0.05 ^{c}	+15.20±0.15 ^{b}	4.44±0.02 ^{d}	16.51±0.01 ^{b}	67.00±0.02 ^{c}

Data in same column with different letters are significantly different (p<0.05). L* measures luminosity on a scale from 0 to 100 (from black to white); a* indicates (+) red or (-) green; b* indicates (+) yellow or (-) blue; The total color difference (ΔE), chroma (C), hue angle (h)

Table 3: Bioactive properties of control and treated pineapple juices

Treatments	TPC (mg GAE/100 ml)	TAC (mg TA/100 ml)	Antioxidant capacity (µmol TE/ml)
Control	11.803±0.002 ^{c}	0.193±0.002 ^{b}	25.390±0.030 ^{e}
Microwave	11.870±0.020 ^{b}	0.190±0.002 ^{b}	26.075±0.004 ^{b}
Vacuum evaporation	11.856±0.008 ^{b}	0.188±0.001 ^{c}	25.873±0.002 ^{c}
Mild pasteurization	11.586±0.002 ^{d}	0.179±0.002 ^{d}	25.597±0.003 ^{d}
Pulsed electric field	11.140±0.015 ^{e}	0.191±0.002 ^{b}	25.572±0.002 ^{d}
Ultra-sonication	11.996±0.002 ^{a}	0.235±0.001 ^{a}	26.157±0.001 ^{a}

Data in same column with different letters are significantly different (p<0.05). GAE=Gallic Acid Equivalents; TA=Total Anthocyanin; TE=Trolox Equivalent; TPC=Total Phenolic Content; TAC=Total Anthocyanin Content

Table 4: Microbiological properties of control and treated pineapple juices

Treatments	TBC (10^4 CFU/ml)
Control	9.50±0.20 [a]
Microwave	4.70±0.10 [d]
Vacuum evaporation	6.30±0.30 [b]
Mild pasteurization	2.80±0.20 [f]
Pulsed electric field	5.20±0.30 [c]
Ultra-sonication	3.50±0.20 [e]

Data in same column with different letters are significantly different (p<0.05).
CFU=Colony Forming Units; TBC=Total Bacterial Count

Discussion

The obtained results demonstrated the influence of non-thermal processing methods on the physicochemical properties of pineapple juices. TSS (°Brix) is one of the determining factors for the quality grading of citrus juice. However, marginal reduction in TSS observed in both ultra-sonication and pulsed electric field treatment might be ascribed to the reduced penetration of soluble solids through cellular membranes. This finding regarding the reduction of TSS is consistent with the findings of Khandpur and Gogate (2016) in sonicated fruits (orange, sweet lime) and vegetable (carrot and spinach) juices. Bhat et al. (2011) also found that sonication reduces the TSS of kasturi lime juice significantly. Results regarding pH clearly stated that obtained values are within the recommended pH level for the pineapple juice (≤4.5), so as to retaining the optimum quality during storage (Ali et al., 2015). Furthermore, there was no statistically significant difference in pH values between microwave processing and mild pasteurization treatment. Vacuum evaporation and ultra-sonication of pineapple juice produced comparatively higher and lower pH values, respectively. These findings are consistent with previously reported findings for an orange-carrot juice blend (Rivas et al., 2006). However, the cavitation and shearing effects of ultra-sonication results to unfolding and refolding of particles which might release more protons (H^+), thereby accompanied the acidic pH (Zhao et al., 2022). Similarly, mild pasteurization increased the titratable acidity of pineapple juice, while ultra-sonication produced the least acidity value. Rivas et al. (2006) found an increase in titratable acidity in pasteurized orange and carrot juice. Furthermore, Zárate-Rodríguez et al. (2000) found no differences in titratable acidity of PEF-treated apple juice when compared to an untreated sample, which is consistent with the current study's findings. However, the use of high power sonication and the decomposition of water inside the cavities might result in the formation of free radicals such as H+ and OH radicals, which could be responsible for the increased acidity of pineapple juice under the action of ultra-sonication treatment (Cansino et al., 2013).

Food color influences consumer perception of juice quality and is an important factor in determining consumer preferences. The highest L* value was obtained in the ultra-sonication treatment of pineapple juices. The outcome is consistent with the observations of sonicated kasturi lime juice (Bhat et al., 2011). Rawson et al. (2011) also observed the similar effects, reporting that precipitation of unstable particles in the juice due to sonication treatment might be responsible for the increase in L* value. In contrast, in pulsed electric field treatment, non-enzymatic browning reaction accompanied by degradation of carotenoids, anthocyanins, vitamins, and other components in juice decreased the L* value. Aguiló-Aguayo et al. (2009) found that strawberry juice subjected to heat treatment had lower L* values. Rivas et al. (2006) also reported a decrease in L* values in thermally pasteurized orange and carrot juice.

Color intensity, including redness (a*) and yellowness (b*), was higher in microwave processed pineapple juice than in all other treatments studied. In contrast, significant decrease in a* and b* values for vacuum evaporation confirmed significant loss in pineapple juice redness, which can be attributed to the loss of active carotenoids and further indicates quality deterioration. Similar results reported for the effect of both the microwave process and evaporation on the Hunter a* and b* value of pomegranate juice (Maskan, 2006), where they concluded that the presence of sugar and sugar derived residue compounds could have enriched the degradation of anthocyanin compounds and non-enzymatic browning reactions. Moreover, microwave processing had the highest hue angle, followed by ultra-sonication, pulsed electric field, vacuum evaporation, and mild pasteurization. Similarly, Turfan et al. (2011) concluded that the presence of high tannin content combined with heat treatment resulted in higher hue angle in microwave concentrated pomegranate juice.

Furthermore, the characterization of color changes in pineapple juices was also evaluated by predicting color difference (ΔE) and chroma (C) values. Chroma (C) quantifies the intensity of color in pineapple juices, whereas ΔE measures the stability of color during storage. It also indicates the level of color saturation or purity; thus, high values indicate high color saturation (Tiwari et al., 2010). The pulsed electric field treatment produced the highest (ΔE) value, followed by vacuum evaporation, microwave, ultra-sonication, and mild pasteurization treatments. When compared to pasteurized samples, pulsed electric field treatment resulted in better color preservation. In this case, microwave treatment produced the most intense color, followed by ultra-sonication, mild pasteurization, pulsed electric field, and vacuum evaporation. A similar observation was also reported by Cortés et al. (2008). Thus, the current study confirmed that non-thermal techniques resulted in very little change in the color attributes of pineapple juice.

Phenolic compounds are the most common phytochemical groups found in fruit juices (Balasundram et al., 2006). Mild pasteurization followed by pulsed electric field treatment significantly reduced the content of phenolic compounds, indicating that phenolic compounds are highly thermo-sensitive. Aguilar-Rosas et al. (2007) investigated the effect of a pulsed electric field on apple juice pasteurization. They reported the similar finding that is the reduction of total phenolic compounds of about 15%, when compared to untreated juice. The oxidation of these compounds and the polymerization reaction with proteins might be responsible for the decrease in phenolic content (Liu et al., 2014). Furthermore, radical formation during some electrochemical reactions at the electrode surfaces in the pulsed electric field chamber might degrade the phenolic compounds (Assiry et al., 2006). In contrast, when compared to the control and other treatments, ultra-sonication produced the highest total phenol content in pineapple juice. Abid et al. (2014) reported a similar finding, that total phenolic retention was significantly higher in ultrasound-treated apple juices. Similarly, phenolic compound enhancement in ultrasonically treated red wine was reported as well (Masuzawa et al., 2000). However, significant increases in these phenolic phytonutrients could be attributed to enhanced cell wall disruption caused by cavitation as a result of rapid changes in liquid pressures caused by shear forces exerted during sonication, which could lead to the release of some chemically bound polyphenolic phytonutrients and thus increased their availability in the juice.

Anthocyanins are water-soluble colored pigments that are thought to be among the most important health-promoting compounds (Kamrunnaher et al., 2019). These compounds, on the other hand, are easily dissolved in water and can be further broken down by thermal treatment (Patras et al., 2010). The current study found that mild pasteurization significantly reduces the anthocyanin content of pineapple juice. Vegara et al. (2013) also found that pasteurization (65 °C and 90 °C for 30 s or 5 s, respectively) reduced the percentage of anthocyanin in pomegranate juice. The oxidation or condensation of anthocyanin pigments during this treatment may accelerate the anthocyanin degradation (Mahmoud et al., 2017). Due to their non-thermal properties, ultra-sonication treatment produced the highest anthocyanin content in this current study. This finding is consistent with the findings of Tiwari et al. (2008), who reported an increase in the anthocyanin pigment pelargonidin-3-glucoside, which produces the characteristic orange color in strawberry juice. In fact, ultra-sonication treatment generates low temperature heating and allows for rapid mass transfer, which could explain why the highest anthocyanin content was retained.

However, antioxidant capacity results showed that ultra-sonication followed by microwave processing, vacuum evaporation, mild pasteurization, and pulsed electric field treatments were effective in retaining antioxidant capacity of pineapple juices at higher levels. Abid et al. (2014) found that power ultrasound increases DPPH antioxidant activity by 15% in apple juice (*Malus domestica* cv. Fuji). However, increased phenolic content as well as cell wall rupture caused by cavitation during sonication may improve the extraction and availability of these compounds. Furthermore, sonication treatment might increase polyphenolic oxidase activity, which could explain the increase in phytonutrients (Abid et al., 2013). Apart from ultra-sonication treatment, other non-thermal processing methods also demonstrated the increased antioxidant capacity when compared to the control. Hojjatpanah et al. (2011), Sabanci et al. (2019), and Yousuf et al. (2020) reported similar findings on the improved antioxidant capacity of mulberry, pomegranate, and pineapple juices when microwave processing, vacuum evaporation, and pulsed electric field treatment were used instead of conventional thermal processing. Hence ultra-sonication could play an important role for the improvement of total antioxidant capacity in pineapple juice, which is a great advantage of this technique.

Food processing and preservation rely on the removal of microorganisms to extend shelf life and maintain optimum quality. In terms of the microbial properties of pineapple juices, mild pasteurization was found to be very effective for microbial inactivation, resulting in the greatest microbial count reduction, followed by ultra-sonication, microwave processing, pulsed electric field treatment, and vacuum evaporation. Saeeduddin et al. (2015) investigated the impact of ultrasound and pasteurization processing on pear juice, and concluded that pasteurization at 65 °C resulted in a significant reduction in microbial load when compared to the control. Kiang et al. (2013) also reported similar findings, demonstrating that both ultra-sonication and pasteurization treatments reduce the microbial load of mango juice the most. Ultrasound pasteurization of pineapple, cranberry, and grapefruit juices successfully achieved significant pathogenic inactivation (Bermúdez-Aguirre and Barbosa-Cánovas, 2012). However, the reduction in microbial load might be attributed to the enhancement of biocides by cavitations produced during ultra-sonication, which increases localized pressure and temperature without increasing the overall product temperature. Besides, it also produces hydroxyl radicals and shock waves, which ultimately cause microbial inactivation (Bermúdez-Aguirre and Barbosa-Cánovas, 2012; Wordon et al., 2012). Therefore, complete decontamination of pineapple juice could be achieved by the combined effect of mild pasteurization and sonication to get safe fruit juices free from pathogenic microbes with minimal damage to the nutrients and sensory attributes.

Conclusion

This study ensured the perfect phenolic content, antioxidant capacity, retention of anthocyanin content, and attractive color in pineapple juices when treated with non-thermal techniques. Furthermore, the physicochemical properties obtained from treated pineapple juices were satisfactory. As a result, the use of non-thermal technologies in pineapple juice production to improve nutritional quality of the final product is very promising. Future research should concentrate on optimizing non-thermal processing conditions during food processing.

Author contributions

T.A. and N.S. designed the study; J.F. and T.H. conducted the experimental work; R.T. analyzed the data; N.R. wrote the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

There is no conflict of interest in this study.

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