CHAPTER 5

EFFECT OF DIFFERENT PASTEURISATION METHODS ON PHYTOCHEMICAL AND ANTIOXIDANT PROPERTY OF FIVE FRUIT JUICES

5.1. Introduction

Fruits and fruit juices are considered to be highly nutritious in nature and are generally consumed in fresh forms. They are considered as good sources of minerals, vitamins, carotenoids, polyphenols and dietary fibre. [1] But technological advancement in the food processing industries has led to the development of different processing techniques to increase the shelf life of the fruits and fruit products to ensure the availability throughout the year even for the seasonal ones. Number of fruit juice-based products is available in the market. The common processing method for fruit juices is the conventional thermal pasteurisation where high temperature treatment is given for a particular period of time for microbial inactivation that enhances the shelf life. But studies have reported the adverse effect of thermal pasteurisation on the organoleptic quality and bioactive properties in juice of oranges, strawberry and watermelon. [2, 3] Therefore, in order to develop a better preserved juice product, alternate processing methods have been explored and developed. Microwave and ultrasound treatments are two alternate processing methods that are being applied for juice processing in place of conventional thermal pasteurisation.

Microwaves are electromagnetic radiation waves whose frequency lies between infrared and radio and TV waves. The principle of application of microwave radiation to juice pasteurisation is that, water present in food materials acts as an electric dipole i.e., it contains both positively and negatively charged molecules. When an electromagnetic radiation is passed through the food, heat energy is produced due to intermolecular frictions resulting from the movement of electrical charges produced by forces of attraction and repulsion. [4] Another mechanism of heating is due to ionic conduction. The application of electromagnetic field causes migration of the ions towards oppositely charged regions. This results in release of heat due to multiple billiard ball-like collisions and disruption of the H-bonds in water. [5] Tang [6] had suggested that the amount of heat produced in food is proportional to its dielectric properties. Unlike conventional thermal pasteurisation,

microwave heating causes uniform heating of the entire volume of the food and thus, in products like fruit juices with high water content, the energy is absorbed very fast, which causes rapid heating. [5] The rapid heating and significant reduction in time of heat exposure to the product lowers the loss of organoleptic quality and the rate of destruction of heat labile nutrients and bioactive compounds. [7, 8]

Ultrasound processing commonly called as sonication, is a non thermal processing method applied in fruit juice processing. Ultrasound at low frequencies (20-100 KHz) propagates in liquid medium and results in cavitation which involves formation and collapse of bubbles. It results in significant microbial inactivation, improved functionalities and minimal degradation in the quality parameters. [9, 10] Sonication is simple and less time consuming with improved efficiency. [11, 12]

However, depending on the type of fruit juice sample and their bioactive compositions, different processing methods have different effects. Santhirasegaram et al. [13] compared the effect of thermal treatment and sonication on quality attributes of mango juice. Similarly, Igual et al. [7] studied the effect of conventional pasteurisation and microwave treatment in grapefruit juice.

Therefore, a comparative study on the effect of conventional thermal pasteurisation, microwave and ultrasound treatments on the phytochemical and antioxidant activities of juices from carambola (*Averrhoa carambola L.)*, black jamun *(Syzygium cumuni* L.Skeels.), watermelon (*Citrullus lanatus* var *lanatus*), pineapple *(Ananas comosus L.* Merr*)*, and litchi (*Litchi chinensis* Sonn.) was done. The outcome of the study is reported in this chapter.

5.2. Materials and methods

All the chemicals used were of analytical grade and supplied by Merck, India and Himedia Laboratories and Sigma chemicals, India.

5.2.1. Materials

The fruit samples viz. carambola (*Averrhoa* carambola *L.)*, black jamun *(Syzygium cumuni* L.Skeels.), watermelon (*Citrullus lanatus* var *lanatus*), pineapple *(*Ananas comosus *L. Merr)*, and litchi (*Litchi chinensis* Sonn.) were procured from the local fruit market, Tezpur, Assam during the season. The fruits selected for this study were chosen from the thirteen

studied fruits reported in chapter 3 based on their easy availability and suitability for juice extraction.

5.2.2. Fruit juice preparation

The fruit samples were washed and sorted properly and the juice was extracted using a household juicer (Philips juicer). The juice was strained through a muslin cloth and then divided into five lots according to their processing methods as described.

Freshly squeezed fruit juice (FR): The extracted juice was filtered and kept aside separately.

Conventional thermally pasteurized juice (PS): Hundred millilitres of FR was heated in glass tube in a thermostatic water bath (Voltam, India) at 75 ±1°C for 3 min.

Microwave-pasteurized juice (M600W and M900W): Twenty mililitres of FR was heated in 25 mL glass tube at 600W and 900W for 30 s in a microwave oven (Samsung model, India). In these conditions, the samples reached 75 ±1°C at 600W and 80 ±1°C at 900W power level, respectively. [7]

Sonicated fruit juice (SN): Hundred millilitres of FR was sonicated in an ultrasonic cleaning bath for 30 min at 50 ±1°C.

All the treated samples were cooled immediately to 30°C and then stored at -20°C until further analysis.

5.2.3. Total plate count of the treated samples

The total aerobic plate count of the juice samples was determined based on the method of Santhirasegaram et al. [13] using plate count agar plates and inactivation of gram negative bacteria was done by using MacConkey agar plates. For this, 1 mL of undiluted fruit juice was placed on the respective agar plates. The plate count and MacConkey agar plates were incubated (Labtech, South Korea) at 35 ±1°C for 48 h. The microbial counts in samples were calculated as colony-forming units (cfu) per millilitre of juice expressed as log (cfu/mL).

cfu per mL= number of colonies/ mL of aliquot plated **Eq. 5.1**

5.2.4. Colour

Colour values (*L, a, b*) were measured using a hunter colour spectrophotometer (Hunter Colour Lab UltrascanVis). The '*L*' value indicates degree of lightness. '*L*' value in

the range between 0-50 indicates darkness and 51-100 indicates lightness. Similarly, *'a'* means measure of red (positive values) and green colour (negative values); *'b'* measures the yellow (positive value) or blue (negative values) colours [14]. The colour change of the samples was determined by comparing the *L, a, b* values of the treated samples with that of the fresh juice sample. The overall colour change (△E) of the samples was calculated according to Santipanichwong and Suphantharika [15].

$$\triangle E = \sqrt{(L_0^* - L_0)^2 + (a_0^* - a)^2 + (b_0^* - b)^2} \qquad \textbf{Eq. 5.2}$$

Where, △E is the overall change in colour; L_0^* is the '*L*' value of fresh juice; L_0 is the '*L*' value of treated juice; a_0^* the '*a*' value of fresh juice; a_0 is the '*a*' value of treated juice; b_0^* is the '*b*' value of fresh juice and b_0 is the '*b*' value of treated juice.

5.2.5. Changes in the phytochemical and antioxidant activity of the processed juice samples

5.2.5.1. *Determination of total phenolic content*

Total phenolic content in the sample extracts was assessed using the Folin–Ciocalteau assay [16] with slight modification. For the analysis, 20 µL each of extract, gallic acid standard or blank were taken in separate test tubes and to each 1.58 mL of distilled water was added, followed by 100 µL of Folin–Ciocalteau reagent, mixed well and within 8 min, 300 µL of sodium carbonate was added. The samples were vortexed immediately and the tubes were incubated in the dark for 30 min at 40°C. The absorbance was then measured at 765 nm in a UV-Vis spectrophotometer (Cecil, Aquarius7400). The results were expressed in mg GAE/ 100 mL.

5.2.5.2. *Determination of total flavonoid content*

The flavonoid content was determined by aluminium trichloride method. [17] Briefly, 0.5 mL of the extract was mixed with 1.5 mL of 95% ethanol, 0.1mL of 10% aluminium trichloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of deionised water. After incubation at room temperature for 40 min, the reaction mixture absorbance was measured at 415 nm against deionised water blank in a UV-Vis spectrophotometer (Cecil, Aquarius 7400). Results were expressed as quercetin equivalent (mgQE/100 mL) of sample.

5.2.5.3. *Determination of ferric reducing antioxidant property (FRAP)*

FRAP activity of the samples was measured by the method of Benzie and Strain. [18] Briefly, a 40 μL aliquot of properly diluted sample extract was mixed with 3 mL of FRAP solution. The reaction mixture was incubated at 37°C for 4 min and the absorbance was determined at 593 nm in a UV-Vis spectrophotometer (Cecil, Aquarius 7400) against a blank that was prepared using distilled water. FRAP solution was pre warmed at 37°C and prepared freshly by mixing 2.5 mL of a 10 mM 2,4,6-TPTZ [2,4,6-tri(2-pyridyl)-1,3,5-triazine] solution in 40 mM hydrochloric acid with 2.5 mL of 20 mM ferric chloride and 25 mL of 0.3M acetate buffer (pH 3.6). A calibration curve was prepared, using an aqueous solution of ferrous sulfate (1-10 mM). FRAP values were expressed as μM of ferrous equivalent Fe (II) per 100 mL of sample.

5.2.5.4. *Determination of DPPH radical scavenging activity*

Radical scavenging activity of the sample extracts was measured by determining the inhibition rate of DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical. [19] Precisely, 100 μL of extracts was added to 1.4 mL DPPH radical methanolic solution (10^{-4} M). The absorbance at 517 nm was measured at 30 min against blank (100 μL methanol in 1.4 mL of DPPH radical solution) using a UV-Vis Spectrophotometer (Cecil Aquarius 7400). The results were expressed in terms of radical scavenging activity.

Radical scavenging acitivity (%) = [(Ao-As)/ Ao] × 100 **Eq. 5.3**

Where, Ao is absorbance of control blank, and As is absorbance of sample extract.

5.2.6. HPLC study of the polyphenols

Sample Preparation

The juice samples were centrifuged at 3000 rpm for 15 min. The juice supernatant was then filtered through a membrane filter (0.22 μm) before injection.

RP-HPLC (Waters) gradient elution method was used to identify the major phenolic acid composition of the polyphenol extract. Symmetry 300™ C_{18} (5 μm, 4.6 X 250 mm) column with a binary pump (Waters, 1525) and a UV-Vis detector (Waters, 2489) was used. The ethanolic extract was evaporated under vacuum and then redissolved in 1mL methanol. Mobile phases used were acidified ultrapure water (0.1% acetic acid, pH 3.2, mobile phase A) and methanol (mobile phase B). The gradient method: 80 % A (0-8 min), 65 % A (9-12

min), 45 % A (13-16 min), 30 % A (17-20 min), 20 % A (21-30 min), 10 % of A (31-34 min) and then washing of the column with 65 % A (35-39 min) and lastly, 80 % A (40-45 min) was followed. Sample volume of 20 μL was used. The flow rate was maintained at 0.8mL/min and wavelengths used for UV-Vis detector were 254 nm and 325 nm. The standards used for comparison and identification were (+) catechin, caffeic acid, coumaric acid, gallic acid, syringic acid, ferulic acid, chlorogenic acid, rutin hydrate, kaempferol and quercetin. The gallic acid and syringic acid belong to the hydroxybenzoic acid group of phenolic acids whereas ferulic acid, chlorogenic acid, coumaric acid and caffeic acid were the hydoxycinnamic acid derivatives. Catechin, rutin and quercetin were the members of the flavonoid group.

5.2.7. Statistical analysis

All experiments were carried out at least in triplicates and reported as mean ± standard deviation of mean (S.E.M). The data were statistically analyzed by Duncan's multiple range test at $p \leq 0.05$ significant levels using SPSS version 11.5.

5.3. Results and discussion

5.3.1. Microbial load in the processed juice samples

The processed juice samples showed significant reduction in the microbial count (Table 5.1). The total plate or aerobic count study of the treated juice sample showed varied results depending on the treatment method applied. In all the juice samples pasteurised by conventional method and microwaved at 900W, no detection of aerobic microbial colony was observed. Microwave treatment at 600W and sonication treatment revealed that treated juice allowed aerobic microbial growth as 3 log cfu/mL of aerobic microbial colonies were formed. Similarly, microwave treated (600W) pineapple juice recorded 2 log cfu/mL of aerobic microbial colonies. All the processed samples showed no colony growth in MacConkey agar plates and thus can be inferred that gram negative bacteria were absent in the studied samples. The results obtained in case of thermally pasteurized samples are in agreement with the results reported by Rivas et al. [20] The exposure to heat can cause disruption of the cell membrane and damages to the nucleic acids that destroy the microbes. In sonicated samples, ultrasound causes formation of cavitation induced micro bubbles and their collapse resulting in a localized decontamination effect. [21, 22] Valero et al. [23] reported

that sonication is effective in reducing the microbial count of food borne pathogens in orange juice. Previous studies of sonicated cranberry, pineapple and grapefruit juices showed highest inactivation of pathogens. [24] However, in some cases to achieve complete decontamination, sonication treatment needs to be combined with heat. [25]

Table. 5.1. Effect of processing on the microbial load of the juice samples

Treatment	Total plate count (logCFU/mL)	MacConkey platecount (logCFU/mL)
Conventionally pasteurised		
Carambola	ND	ND
Black jamun	ND	ND
Watermelon	ND	ND
Litchi	ND	ND
Pineapple	ND	ND
Microwaved at 600W		
Carambola	ND	ND
Black jamun	2.0	ND
Watermelon	ND	ND
Litchi	3.0	ND
Pineapple	ND	ND
Microwaved at 900W		
Carambola	ND	ND
Black jamun	ND	ND
Watermelon	ND	ND
Litchi	ND	ND
Pineapple	ND	ND
Sonicated		
Carambola	ND	ND
Black jamun	ND	ND
Watermelon	ND	ND
Litchi	3.0	ND
Pineapple	ND	ND

*ND- Not detected; CFU- Colony forming unit

5.3.2. Colour values of the juice samples and overall change in colour (△E) after processing

The colour values of the juice samples and their changes upon processing are presented in Table 5.2. In carambola, black jamun and pineapple, the '*L*' values decreased upon processing, while an increase was observed in watermelon and litchi juices. Similarly, the '*a*' and '*b*' values also significantly decreased in most of the cases with some exceptions.

Among the processing treatments, the overall colour change in carambola juice was more in sonicated (SN) samples followed by thermally pasteurized (PS) and microwave treated juice at 600W (M600W). In black jamun, the colour change was higher for microwaved (M600W & M900W) and thermally pasteurized (PS) samples.

Table 5.2. Comparison of *L a b* values and overall colour changes (△E) after processing

Parameters	FR	PS	M600W	M900W	SN
Carambola					
L	23.86±0.21c	24.36±0.04^{c}	20.59±0.08^{a}	21.84±0.09^{b}	20.09±0.03^{a}
A	0.02±0.001^{b}	-0.03±0.006^{a}	0.19±0.04^{c}	0.35±0.02^{e}	0.21±0.02^{d}
B	0.62±0.02^{c}	0.29±0.03^{b}	1.45±0.01^{d}	1.77±0.07^{d}	-1.34±0.05^{a}
△E	--	0.34±0.06^{b}	3.41±0.07^{b}	2.32±0.05^{a}	4.25±0.09^{c}
Black jamun					
L	24.55±0.11^{c}	25.47±0.06^{c}	19.68±0.15^{b}	11.56±0.17^{a}	20.17±0.04^{b}
A	8.96±0.09^{c}	10.32±0.10^{d}	0.49±0.04^{a}	0.38±0.03^{a}	2.47±0.01^{b}
B	-4.76±0.03^{c}	-4.83±0.05^{c}	-0.33±0.01^{a}	-0.54±0.01^{a}	2.68±0.03^{b}
△E	--	9.73±0.06^{b}	10.07±0.08^{b}	11.56±0.03^{c}	8.10±0.07^{a}
Watermelon					
L	18.28±0.14^{a}	22.19±0.08^{c}	20.57±0.06^{b}	23.65±0.04^{d}	21.26±0.15^{b}
A	1.38±0.03^{c}	0.18±0.02^{b}	0.02±0.006^{a}	0.09±0.01^{a}	0.03±0.002^{a}
B	1.02±0.01^{b}	1.55±0.14^{b}	0.70±0.02^{a}	0.74±0.02^{a}	0.69±0.01^{a}
△E	--	4.05±0.03^{b}	2.06±0.01^{a}	5.53±0.01^{c}	3.29±0.01^{b}
Litchi					
L	31.86±0.09^{a}	38.99±0.18^{c}	29.41±0.14^{a}	36.51±0.07^{b}	37.58±0.17^{b}
A	-1.13±0.04^{b}	-1.09±0.03^{b}	-1.03±0.05^{b}	-1.28±0.01^{b}	-0.68±0.03^{a}
B	-1.95±0.07^{c}	-0.01±0.005^{a}	-1.38±0.07^{c}	0.71±0.008^{b}	-0.03±0.007^{a}
△E	--	7.72±0.09^{c}	4.66±0.03^{a}	5.38±0.03^{a}	6.32±0.07^{b}
Pineapple					
L	24.59±0.16^{d}	27.34±0.12^{e}	22.03±0.04^{b}	23.27±0.10^{c}	20.94±0.09^{a}
A	-1.31±0.06^{d}	-0.87±0.03^{c}	-0.51±0.09^{b}	-0.18±0.09^{a}	-0.26±0.06^{a}
B	2.35±0.05^{d}	2.14±0.07^{d}	1.26±0.05^{c}	0.92±0.02^{b}	0.41±0.01^{a}
△E	--	3.52±0.01^{b}	3.32±0.01^{b}	2.45±0.05^{a}	4.42±0.03^{c}

*FR-fresh juice; PS- conventional thermal pasteurisation; M600W-microwaved at 600W; M900W-microwaved at 900W and SN- sonicated

Results are mean±S.D of triplicates. Same letter between the bars means no significant difference at p≤0.05 by DMRT.

Similarly, watermelon juice of M900W showed maximum impact on colour change. The colour change in litchi was more in PS and SN samples. However, in sonicated (SN) pineapple maximum change was observed. Overall, processing affected the colour properties of the samples depending on the sample type and processing method used and susceptibility of the natural pigments present in juice samples to the degree and time of their exposure to temperature.

5.3.3. Phytochemical and antioxidant changes

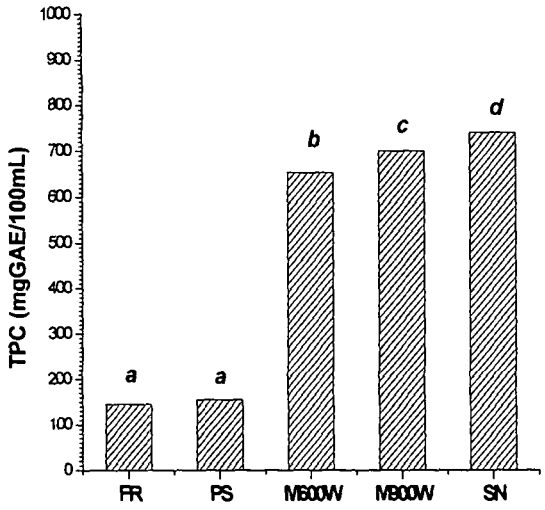
The TPC value of the samples varied depending on the processing treatment applied (Fig. 5.1). In carambola juice, processing caused an increase in phenolics except in PS sample where no change was observed. The highest TPC value was observed in SN sample. Similarly, in watermelon juice, highest TPC was detected in M600W sample. The black jamun PS sample showed highest TPC value, while the microwaved and sonicated sample caused a decrease in TPC. The sonicated litchi showed highest TPC. Microwave (M600W and M900W) and thermal pasteurisation (PS) had a negative impact on the TPC. Lastly, in pineapple juice, the phenolic content in PS and SN sample were slightly lower than the fresh samples but are comparable to each other among themselves, while the highest value was observed in M600W and M900W samples.

The total flavonoid content (TFC) in pineapple was lowered upon processing, whereas in rest of the samples, it showed varied results depending on the processing method employed (Fig. 5.2). Overall, sonication had a positive effect on the TFC in all the juice samples followed by microwave treatment with exceptions in some cases.

The FRAP value of processed carambola juice was higher in thermally pasteurized and microwaved samples (Fig.5.3). In black jamun and pineapple juice, a decrease in reducing property was observed except in PS samples. Similarly, in litchi and watermelon juices, decreases in FRAP values was observed. However, highest decrease in watermelon juice was observed in sonicated samples while, in litchi M900W showed highest decrease.

The DPPH radical scavenging activity also varied among the processed juice samples (Fig 5.4). The DPPH activity was lowest in watermelon juice sample variants (10.53-40.77%) and showed a negative effect on processing. In carambola and black jamun,

DPPH activity ranged between 85.58 % and 97.11% and showed no major difference in their activity upon processing. However, in litchi juice, decreases in microwaved and sonicated samples were detected. The microwaved and sonicated pineapple sample showed increase in DPPH activity.



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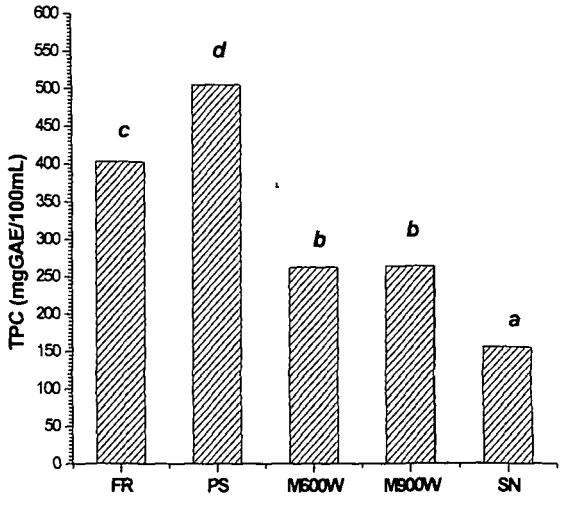

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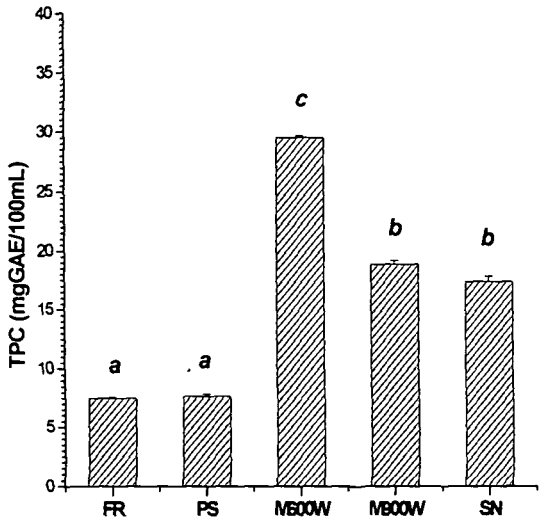

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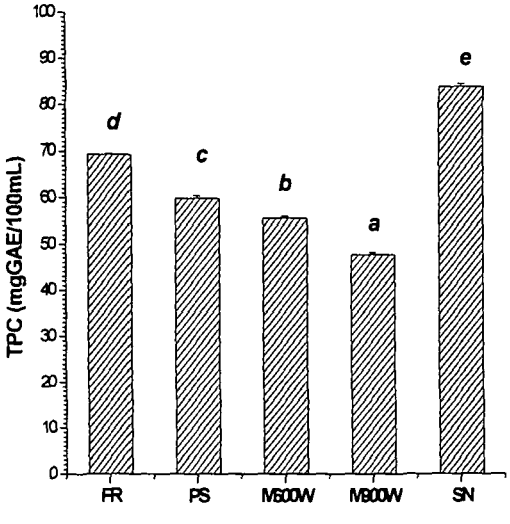

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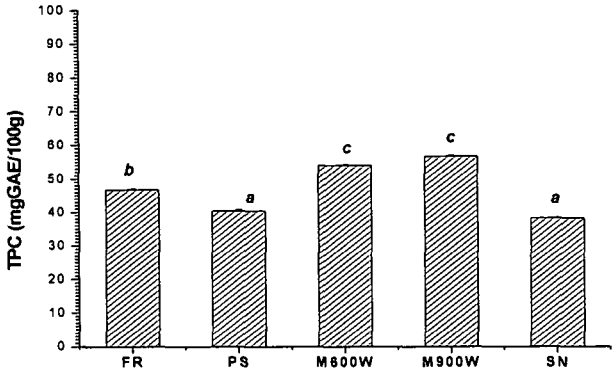
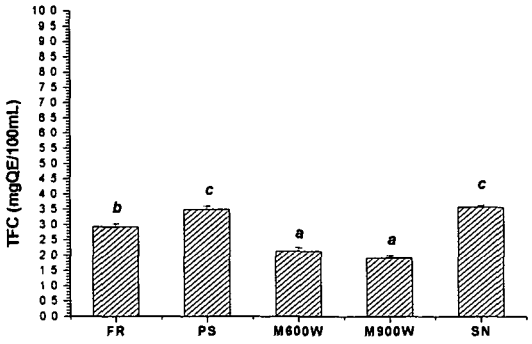


(e)

Fig. 5.1. TPC of five fresh and treated fruit juices. (a) carambola, (b) black jamun, (c) watermelon (d) litchi and (e) pineapple. FR- fresh juice; PS- conventional thermal pasteurisation; M600W- microwaved at 600W; M900W- microwaved at 900W and SN- sonicated

Results are mean±S.D of triplicates. Same letter between the bars means no significant difference at $p \leq 0.05$ by DMRT.



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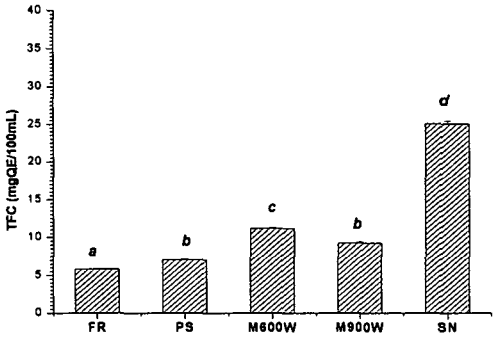

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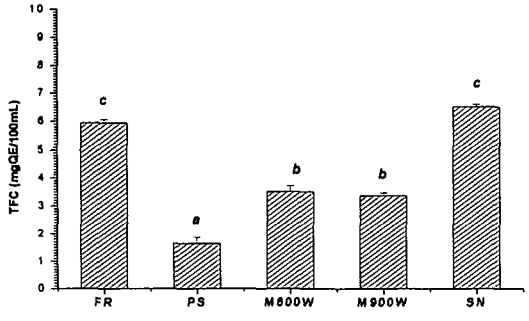

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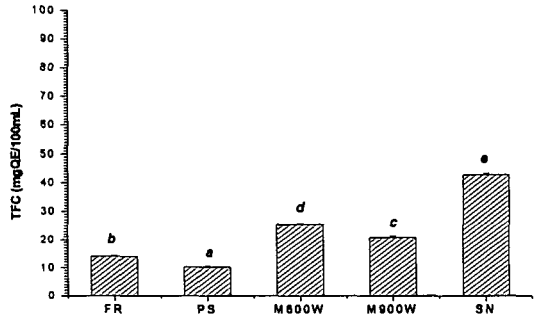

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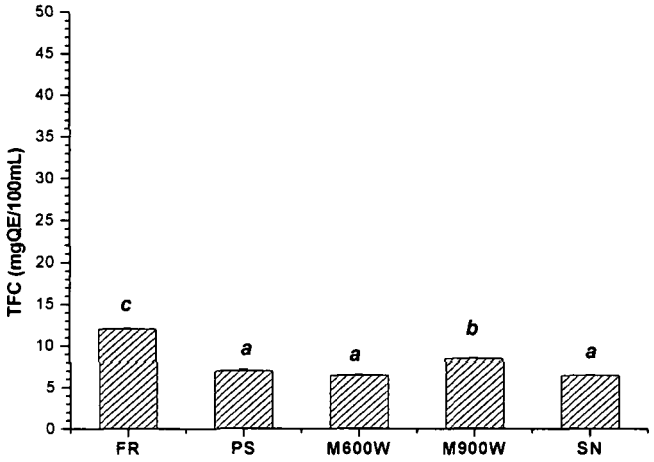
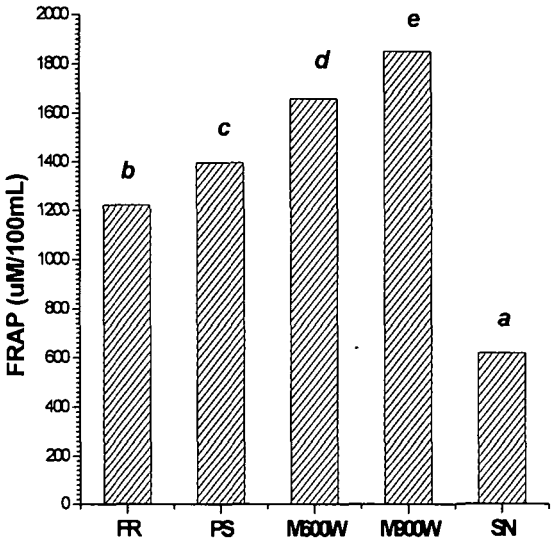


(e)

Fig. 5.2. TFC of five fresh and treated fruit juices. (a) carambola, (b) black jamun, (c) watermelon (d) litchi and (e) pineapple. FR- fresh juice; PS- conventional thermal pasteurisation; M600W- microwaved at 600W; M900W- microwaved at 900W and SN- sonicated

Results are mean±S.D of triplicates. Same letter between the bars means no significant difference at $p \leq 0.05$ by DMRT.



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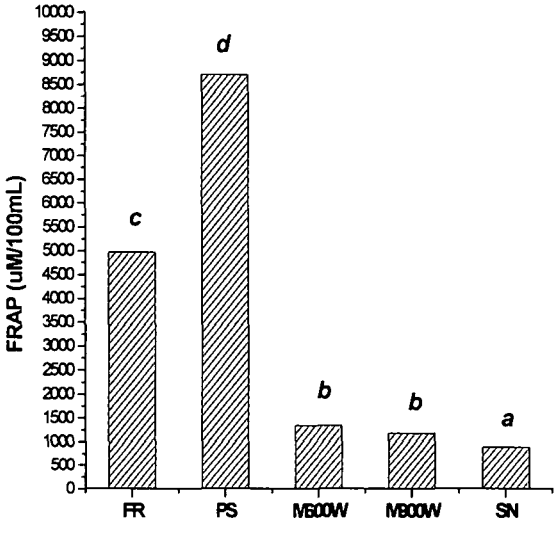

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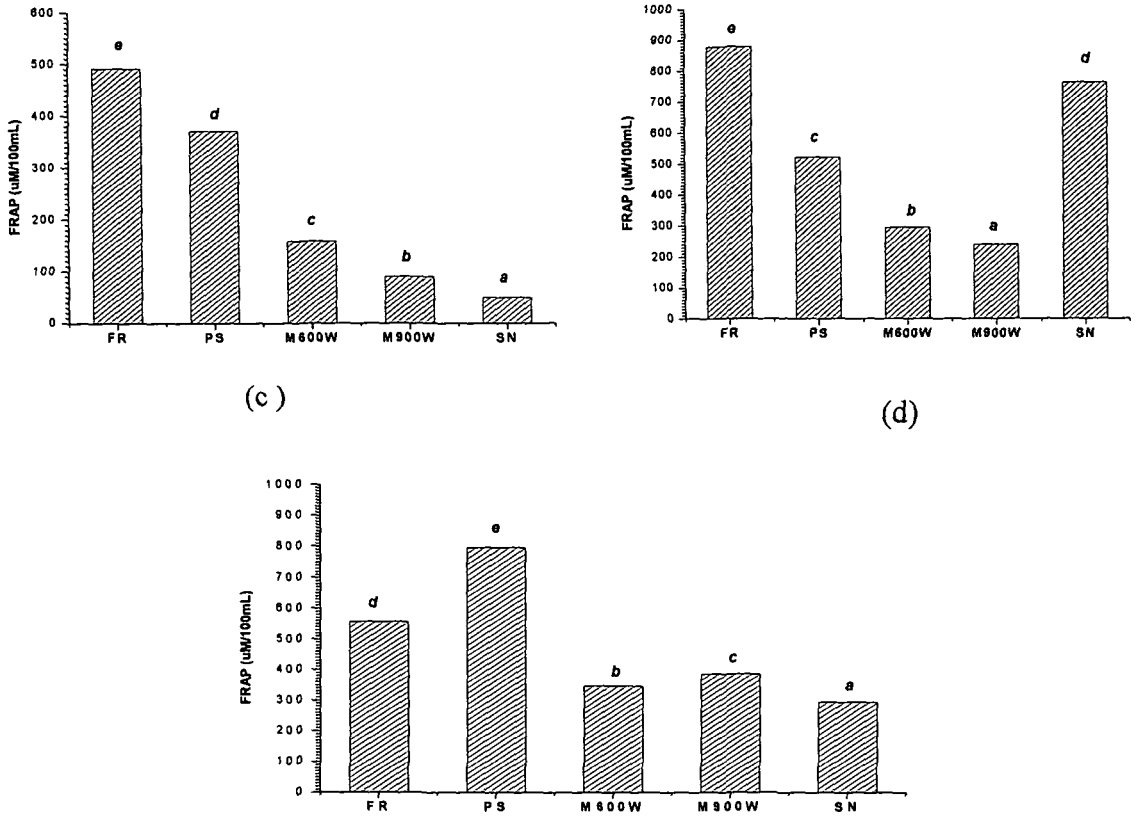
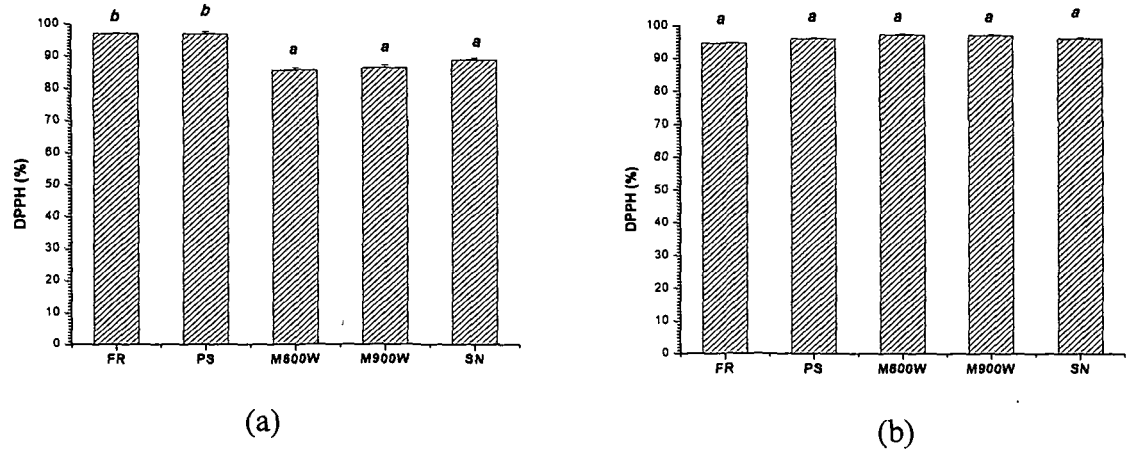
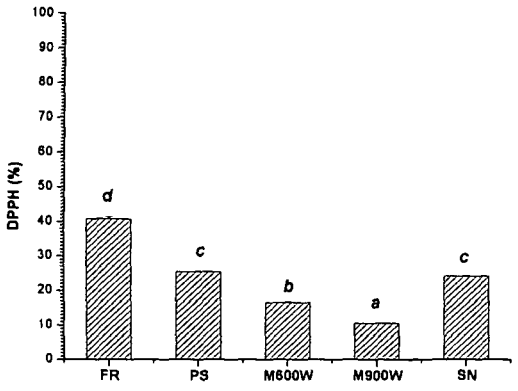
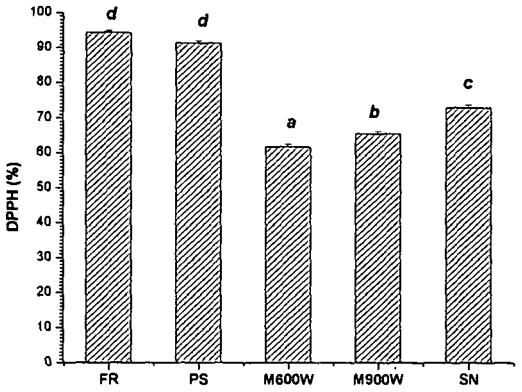


Fig. 5.3. FRAP of five fresh and treated fruit juices. (a) carambola, (b) black jamun, (c) watermelon (d) litchi and (e) pineapple. FR- fresh juice; PS- conventional thermal pasteurisation; M600W- microwaved at 600W; M900W- microwaved at 900W and SN- sonicated. # Results are mean±S.D of triplicates. Same letter between the bars means no significant difference at $p \leq 0.05$ by DMRT.

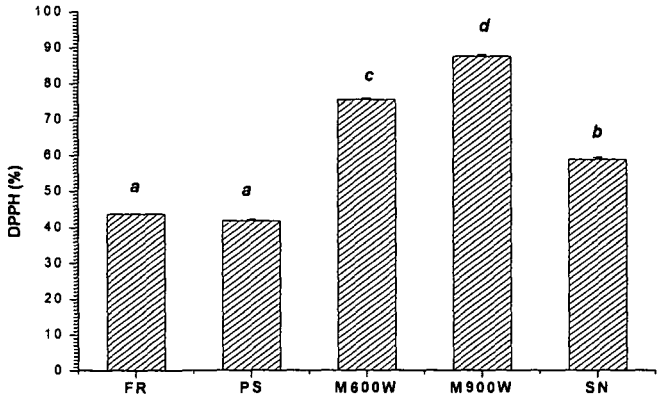




(c)



(d)



(e)

Fig. 5.4. DPPH activity of five fresh and treated fruit juices. (a) carambola, (b) black jamun, (c) watermelon (d) litchi and (e) pineapple. FR- fresh juice; PS- conventional thermal pasteurisation; M600W- microwaved at 600W; M900W- microwaved at 900W and SN- sonicated
Results are mean±S.D of triplicates. Same letter between the bars means no significant difference at $p \leq 0.05$ by DMRT.

The decrease in phytochemicals and antioxidant activity in some cases could be due to destruction of heat labile phenolic compounds present in the juices. [26] But, increased phenolic content in some pasteurized juices could be due to biochemical reactions that could have occurred during heat processing which led to the release of bound phenolics from the fruit matrix and also to the formation of new phenolic compounds by structural rearrangement. [27] Processing might have caused significant effects on cell membranes or in phenolic complexes with other compounds, releasing some free phenolic acids or

flavonoids. [28] Heat might have also inactivated the polyphenol oxidase, preventing further loss of phenolic compounds. But mainly, the increase or decrease in phenolic content depends on the overall composition and types of individual phenolic acid present in maximum in the concerned fruit juice. On heating phenolic compounds have a tendency to undergo some kind of structural rearrangement that could lead to either increased or decreased antioxidant activities.

5.3.4. HPLC determination of the phenolic acids and ascorbic acid content in the processed juice samples

The phenolic acids detected are given in Table 5.3. The following phenolic acids from the obtained chromatograms at 254 nm were compared and identified with their known standards in the fresh and processed fruit juice samples. Gallic acid (RT=3.23 min), catechin (RT=11.89 min), chlorogenic acid (RT=13.54 min), caffeic acid (RT=14.49 min), syringic acid (RT=14.73 min), ferulic acid (RT=16.55), coumaric acid (RT=16.72 min), rutin (RT= 17.31 min), kaempferol (RT=19.61 min) and quercetin (RT=19.89 min). The peak intensities of the chromatogram at 325 nm were very less and hence, only the results obtained at 254 nm had been included and discussed (Fig. 5.5, 5.6, 5.7, 5.8 & 5.9). The phenolic acids in some processed carambola juice samples showed decrease or complete destruction while, in some cases, an increase or appearance of new phenolic acid originally not detected in the fresh juice was observed viz., in PS, M600W and SN juice. In carambola, fresh (FR) and sonicated (SN) juices showed the presence of gallic acid, catechin, chlorogenic acid, syringic acid and ferulic acid. In thermally pasteurized carambola juice, destruction of gallic acid was observed. Similarly, microwaved juice at 600W (M600W) showed absence of both gallic and chlorogenic acid. However, processing had increased the phenolic content in most of the cases.

In black jamun, like carambola, processing increased the gallic acid and syringic acid content in most of the cases. Catechin was not detected in fresh black jamun but both the microwaved juice samples showed good content. Similarly, watermelon juice upon processing showed formation and appearance of newer phenolic acids as well as destruction of some of the existing phenolics of the fresh juice.

The litchi juice showed a decrease in quercetin and rutin which belongs to the flavonoid family and are generally sensitive to heat. [29] However, the microwaved (M600W & M900W) and sonicated (SN) litchi juice showed presence of ferulic acid. On the other hand, sonicated pineapple juice had a negative impact on the gallic acid, chlorogenic acid and quercetin, while it showed release and subsequent detection of catechin, syringic acid, ferulic acid and kaempferol, although in very small quantities.

The destruction of phenolics in most of the cases could be due to heat labile nature of them as well as oxidation due to other factors like light and oxygen. [30] Similarly, the increase and detection of new phenolic acids originally absent in the fresh and unprocessed samples could be the result of release of the bound phenolics. The phenolic acids comprise of both free and bound phenolic acids. The bound phenolic acids remain bound to the some structural carbohydrate and protein either through ester linkage with carboxylic groups or ether linkages with lignin through their hydroxyl groups in the aromatic ring or acetal bonds. [31-34] Application of heat may break these bonds and cause their release due to cell disruption and rupture of the food matrix which in turn facilitates their release in to the liquid medium. [35] The majority of phenolic acids present in citrus fruits are found in their bound form. [36, 37] Increase in the content of some phenolic acid and their antioxidant activity after heat processing has been reported by Kang et al.[38] Similarly, Guihua et al. [39] reported an increase in some phenolic acids such as ferulic acid and chlorogenic acid in some cases after heating. They also reported the decrease in the phenolic acid content with application of heating time and temperature. This might be the result of the cleavage of the esterified bond between sugar glycoside and phenolic acids.

Another probable reason for increase in phenolic content could be due to degradation and molecular rearrangements of the existing phenolic acids during processing. [40] Sonication usually causes leaching and hydrolysis of the free as well as the bound phenolic compounds. [41, 42] Similarly, microwave heating increases the tissue rupture and releases some of the phenolic acids in to the liquid medium or solvent used for extraction in addition to partial degradation of hemicelluloses and lignin that releases the bound phenolics. [43, 44]

Table 5.3. Phenolic acid content in the fresh and processed fruit juice samples

Sample	GA	CTH	CFA	CGA	SA	FA	CMA	RTH	KF	QCT
Carambola										
FR	4.89±0.03	2.90±0.04	ND	2.17±0.06	3.51±0.03	4.21±0.06	ND	ND	ND	ND
PS	ND	4.13±0.02	2.22±0.07	4.23±0.03	7.47±0.12	14.41±0.08	ND	ND	ND	0.66±0.02
M600W	ND	4.07±0.05	2.33±0.05	ND	8.48±0.09	13.96±0.06	2.81±0.02	ND	ND	ND
M900W	11.68±0.05	5.26±0.07	ND	5.15±0.02	9.74±0.07	18.83±0.08	ND	ND	ND	ND
SN	8.94±0.08	3.63±0.06	1.96±0.03	3.63±0.07	6.18±0.05	13.21±0.09	2.36±0.05	ND	ND	0.65±0.03
Black jamun										
FR	40.97±0.14	ND	ND	ND	9.16±0.10	ND	ND	ND	0.72±0.02	ND
PS	16.07±0.12	2.79±0.08	ND	ND	1.67±0.03	ND	2.32±0.01	ND	ND	ND
M600W	67.24±0.17	46.77±0.12	ND	ND	20.08±0.08	ND	ND	ND	ND	ND
M900W	59.01±0.15	31.01±0.05	ND	ND	16.78±0.04	ND	ND	ND	ND	ND
SN	60.18±0.09	ND	2.58±0.01	ND	ND	13.51±0.05	1.78±0.07	0.85±0.01	ND	ND
Watermelon										
FR	0.91±0.03	ND	ND	1.13±0.02	ND	ND	ND	ND	ND	0.46±0.02
PS	5.99±0.09	ND	ND	31.45±0.10	1.05±0.04	ND	1.23±0.07	ND	ND	0.55±0.04
M600W	ND	ND	ND	ND	ND	ND	5.76±0.05	ND	ND	0.33±0.01
M900W	ND	ND	ND	ND	ND	ND	4.52±0.03	ND	ND	0.57±0.02
SN	ND	2.53±0.04	ND	ND	0.87±0.02	ND	ND	ND	ND	ND
Litchi										
FR	12.33±.09	ND	ND	ND	ND	ND	ND	9.11±0.05	ND	2.11±0.07
PS	11.71±0.12	ND	ND	ND	ND	ND	ND	8.31±0.08	ND	1.54±0.05
M600W	ND	ND	ND	ND	ND	19.76±0.11	ND	4.93±0.11	ND	1.14±0.02
M900W	ND	ND	ND	ND	ND	17.91±0.09	ND	4.29±0.04	ND	0.93±0.01
SN	ND	ND	ND	ND	ND	21.33±0.15	ND	5.47±0.14	ND	0.83±0.04
Pineapple										
FR	22.61±0.11	ND	ND	3.86±0.11	ND	ND	ND	ND	ND	2.94±0.03
PS	20.72±0.14	ND	ND	5.77±0.09	ND	ND	ND	ND	ND	2.73±0.11
M600W	9.11±0.08	ND	ND	4.56±0.04	ND	ND	ND	ND	ND	2.27±0.07
M900W	13.90±0.03	ND	ND	4.17±0.07	ND	ND	ND	ND	ND	ND
SN	ND	8.0±0.05	ND	ND	11.95±0.11	6.53±0.16	ND	ND	0.37±0.03	ND

GA- gallic acid; CTH- catechin; CGA-chlorogenic acid; CFA- caffeic acid; SA- syringic acid; FA- ferulic acid; CMA- coumaric acid; RTH- rutin hydrate; KF- Kaempferol; QCT- quercetin

*Results (mg/100 mL) are mean ±S.D of triplicate values; FR- fresh juice; PS- conventional thermal pasteurisation; M600W- microwaved at 600W; M900W- microwaved at 900W and SN- sonicated

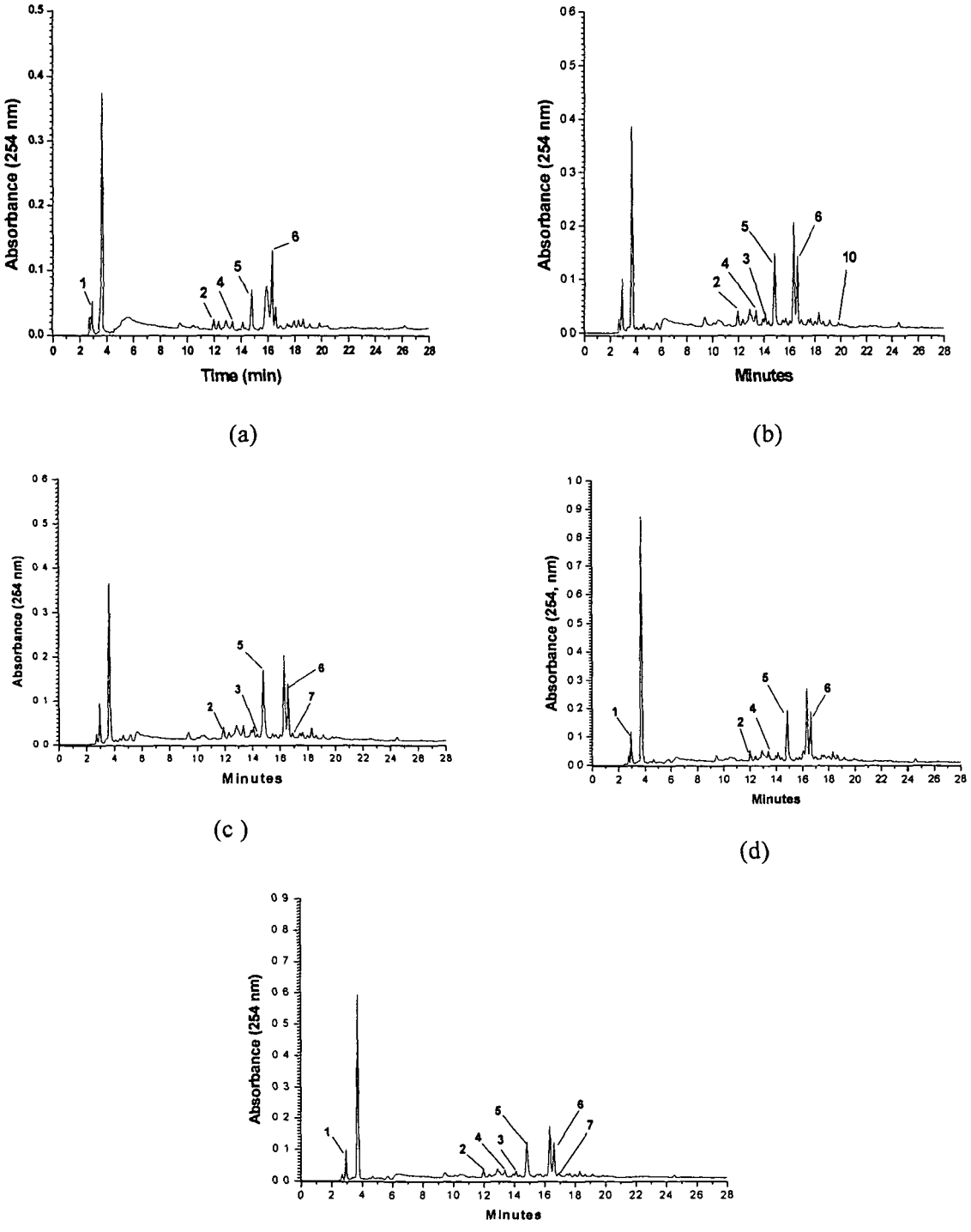


Fig. 5.5. RP-HPLC chromatogram of carambola juices. (a) fresh, (b) pasteurised, (c) microwaved at 600W (d) microwaved at 900W and (e) sonicated at 254nm.

**1=gallic acid; 2= catechin; 3= caffeic acid; 4= chlorogenic acid; 5=syringic acid; 6= ferulic acid; 7= coumaric acid; 8= rutin hydrate; 9= kaempferol and 10= quercetin.

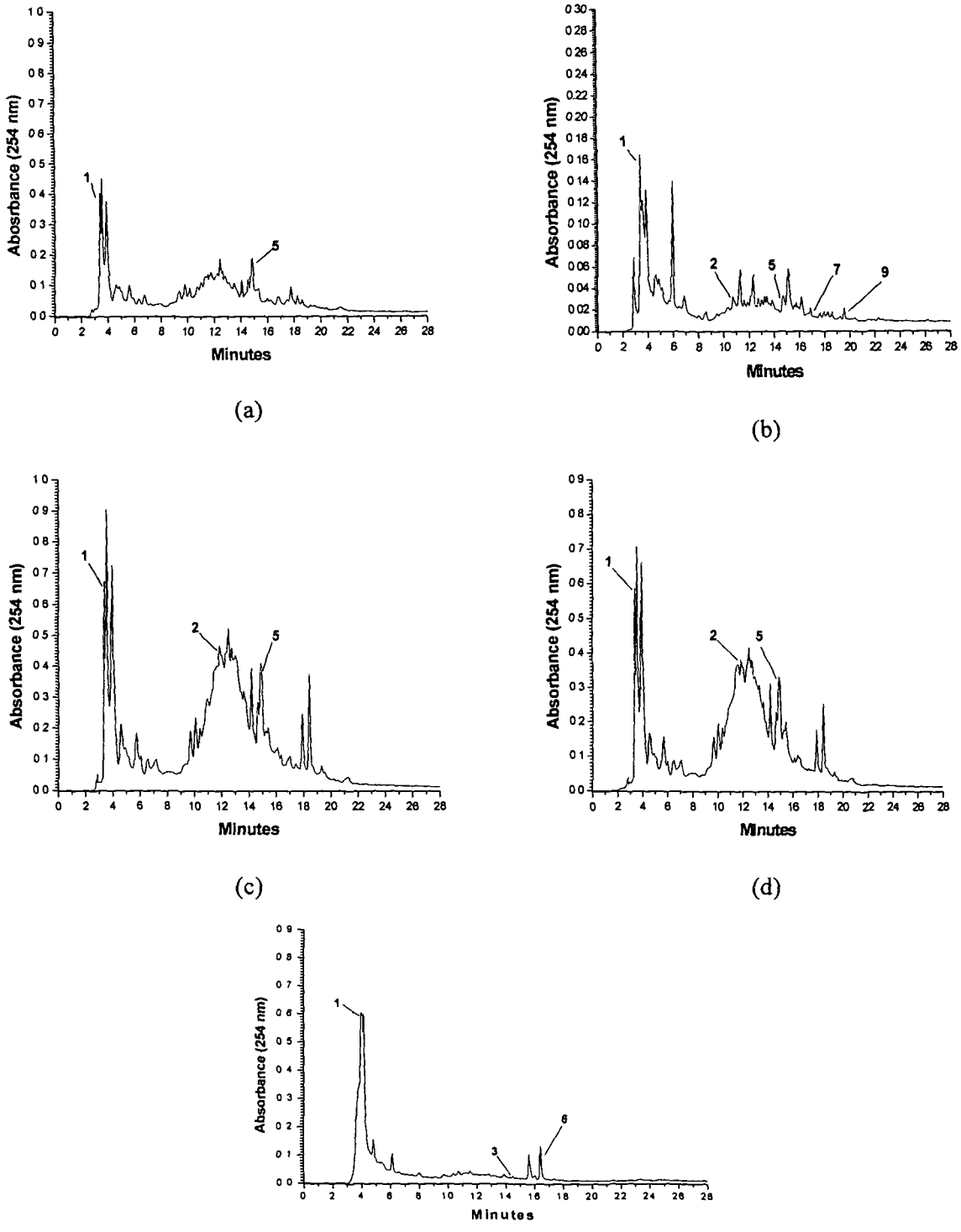


Fig. 5.6. RP-HPLC chromatogram of black jamun juices. (a) fresh, (b) pasteurised, (c) microwaved at 600W (d) microwaved at 900W and (e) sonicated at 254nm.

**1=gallic acid; 2= catechin; 3= caffeic acid; 4= chlorogenic acid; 5=syringic acid; 6= ferulic acid;7= coumaric acid; 8= rutin hydrate; 9= kaempferol and 10= quercetin

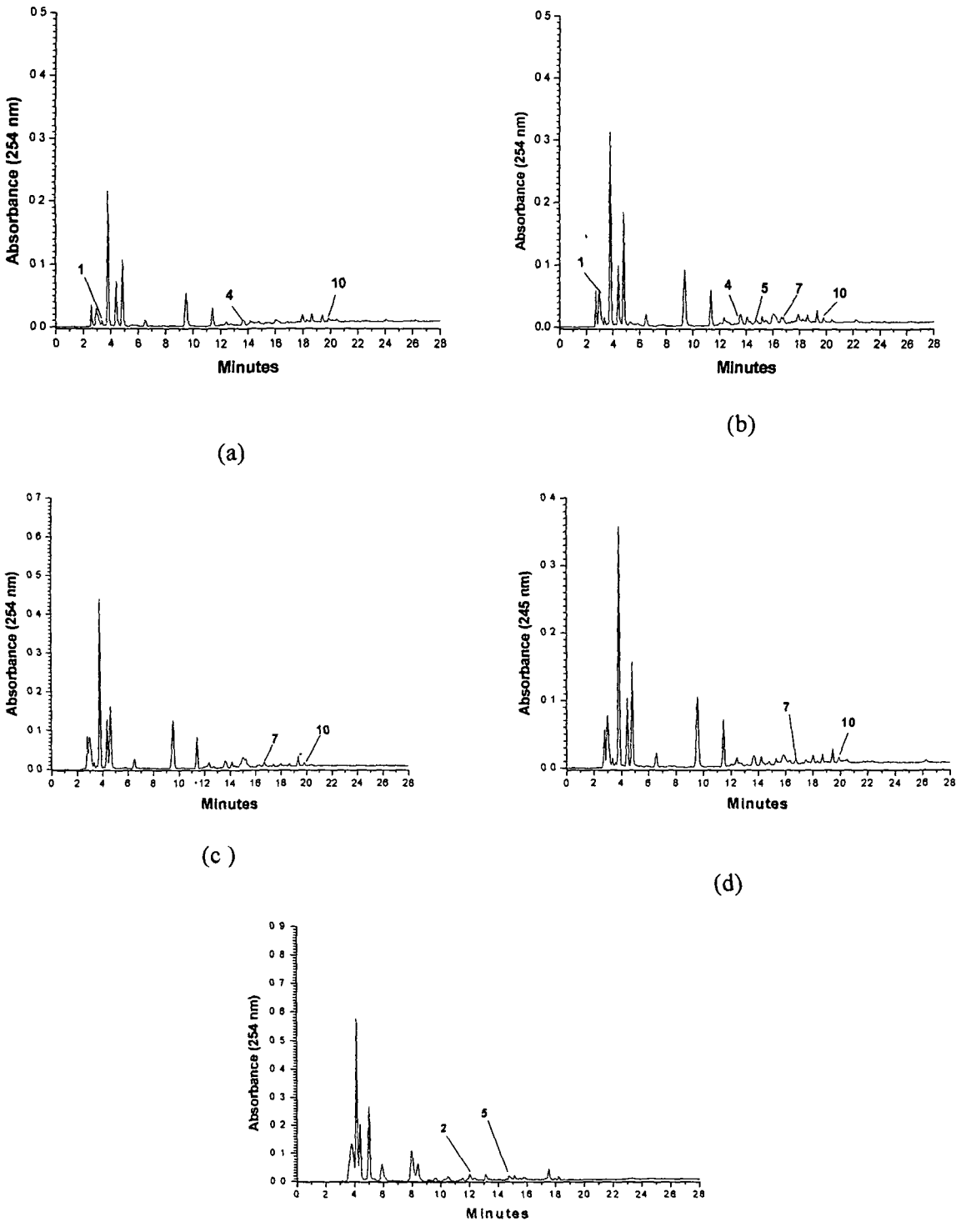


Fig. 5.7. RP-HPLC chromatogram of watermelon juices. (a) fresh, (b) pasteurised, (c) microwaved at 600W (d) microwaved at 900W and (e) sonicated at 254nm.

**1=gallic acid; 2= catechin; 3= caffeic acid; 4= chlorogenic acid; 5=syringic acid; 6= ferulic acid; 7= coumaric acid; 8= rutin hydrate; 9= kaempferol and 10= quercetin

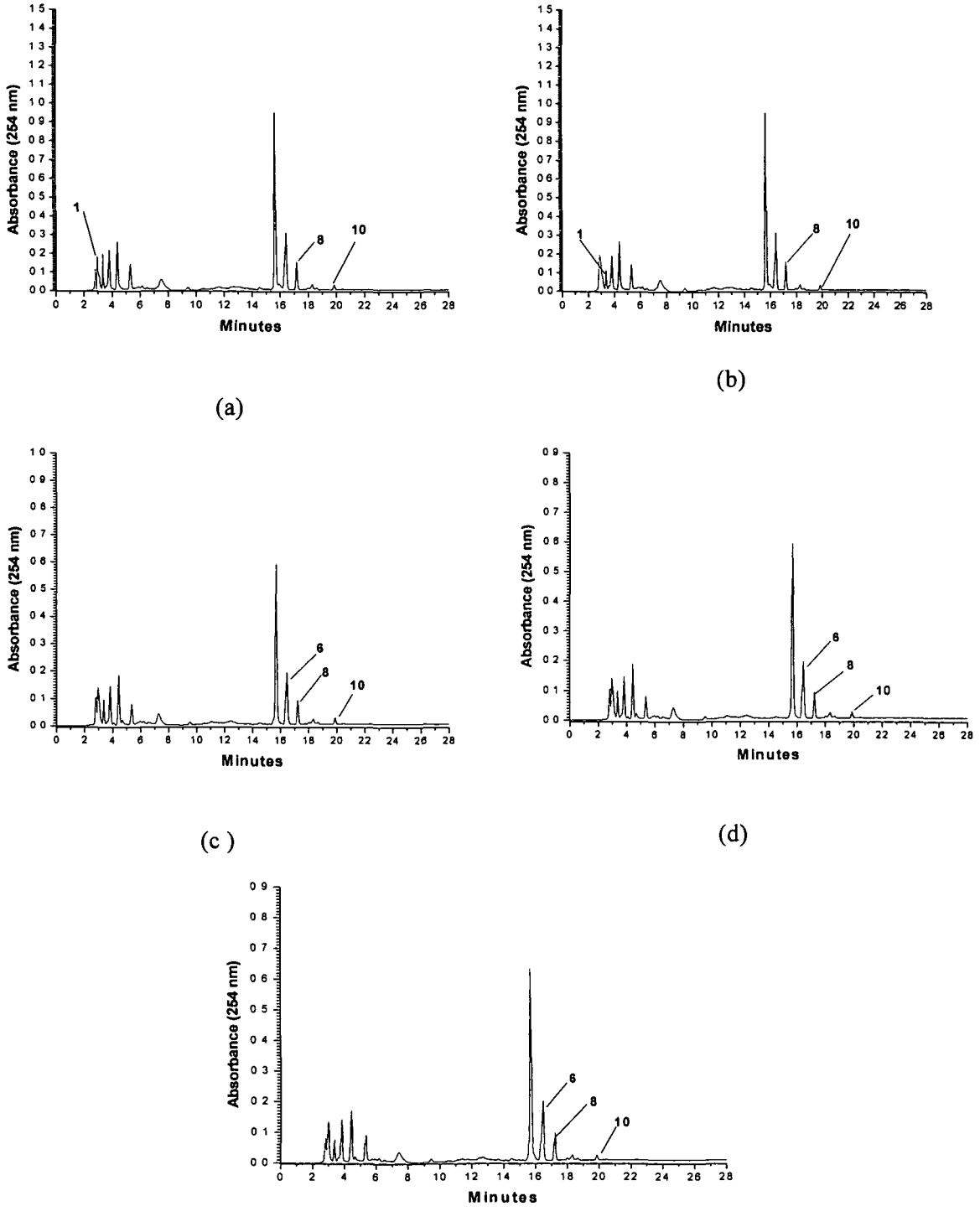


Fig. 5.8. RP-HPLC chromatogram of litchi juices. (a) fresh, (b) pasteurised, (c) microwaved at 600W (d) microwaved at 900W and (e) sonicated at 254nm.

**1=gallic acid; 2= catechin; 3= caffeic acid; 4= chlorogenic acid; 5=syringic acid; 6= ferulic acid; 7= coumaric acid; 8= rutin hydrate; 9= kaempferol and 10= quercetin

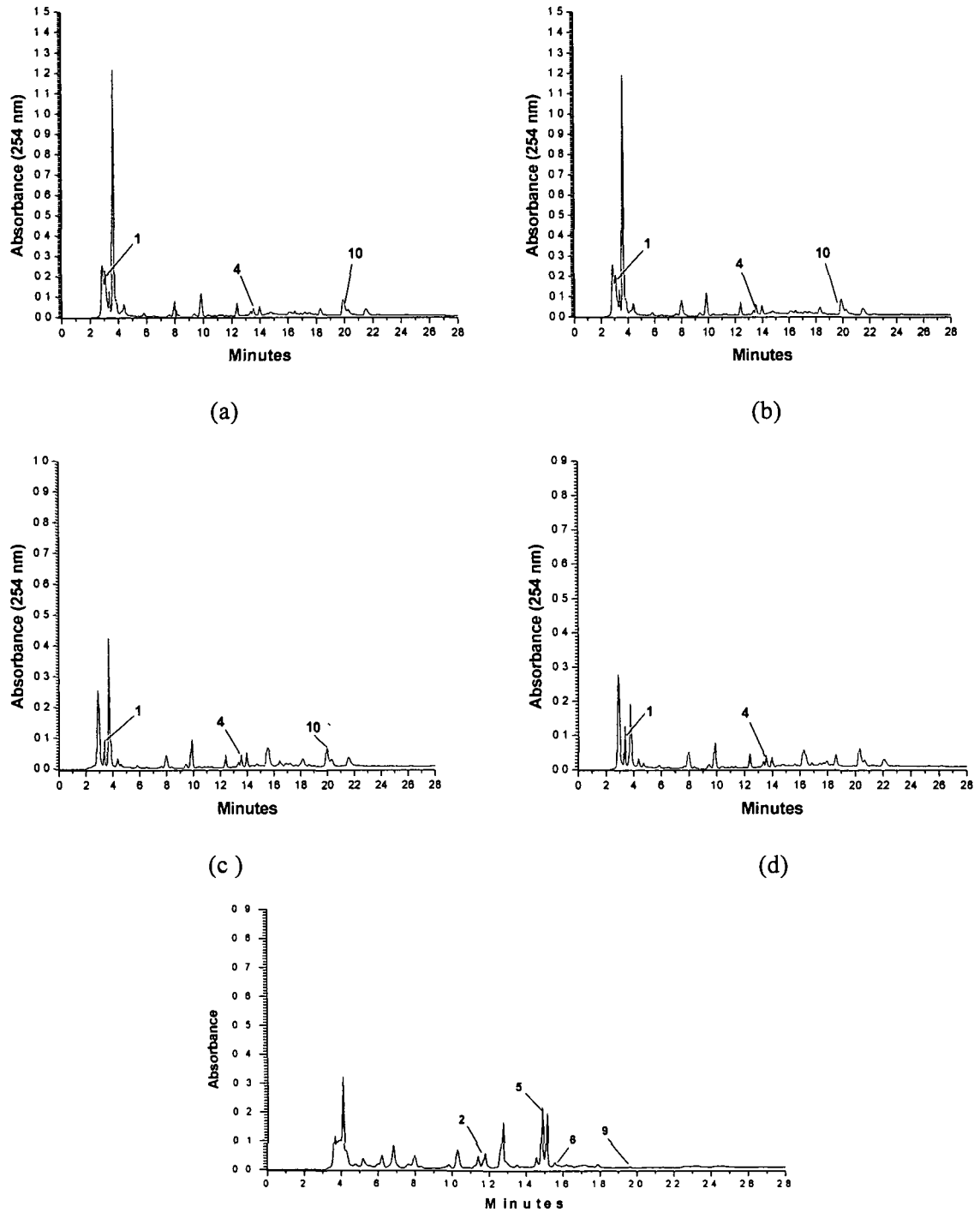


Fig. 5.9. RP-HPLC chromatogram of pineapple juices. (a) fresh, (b) pasteurised, (c) microwaved at 600W (d) microwaved at 900W and (e) sonicated at 254nm.

**1=gallic acid; 2= catechin; 3= caffeic acid; 4= chlorogenic acid; 5=syringic acid; 6= ferulic acid ;7= coumaric acid; 8= rutin hydrate; 9= kaempferol and 10= quercetin

Overall, carambola and litchi juice variants showed good content of ferulic acid. Also, black jamun and pineapple juices are rich in gallic acid as well as rutin and quercetin. Depending on the sample type and phenolic acid compositions, it was found that processing had both positive as well negative impacts on the juice samples. Compared to the thermally pasteurized juice samples, microwave treatment involved less exposure time to high temperature, while sonication involved use of low temperature and both microwaved and sonicated samples were found to increase the phenolic content and antioxidant activity with exception in some cases.

5.4. Conclusions

Microbial inactivation in almost all the processed juice samples was observed. All the processed juice showed no growth in MacConkey agar plates and thus, it can be inferred that no pathogenic gram negative bacteria was present in the samples. In most cases, compared to the conventional thermal pasteurisation, microwaved and sonicated sample showed more positive effect on the phytochemical content. Depending on the type of fruit sample and treatment, increase or decrease in phytochemical values was observed. HPLC study showed the presence of different phenolic acids depending on the sample type and also a few new phenolic acids were detected in some treated samples. The phenolic acids in some processed carambola juice samples showed decrease or complete destruction, while in some cases, an increase or appearance of newer phenolic acid originally not detected in the fresh juice was observed as seen in PS, M600W and SN juices. In carambola, fresh (FR) and sonicated (SN) juices showed the presence of gallic acid, catechin, chlorogenic acid, syringic acid and ferulic acid. The litchi juice showed a decrease in quercetin and rutin. On the other hand, sonicated pineapple juice had a negative impact on the gallic acid, chlorogenic acid and quercetin, while it showed release and subsequent detection of catechin, syringic acid, ferulic acid and kaempferol, although in very small quantity. Microwave treatment involved less exposure time to temperature, while sonication involved use of low temperature and hence, both microwaved and sonicated samples were found to have positive effect on the phenolic content and antioxidant activity with

exceptions in some cases. Therefore, microwave and sonication treatment could be used in place of thermal pasteurisation depending on the sample requirements.

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