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Effect of blanching on the stability of bioactive compounds from *carica papaya* leaves

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Abstract
This study was aimed to determine the effects of blanching process parameters on the extraction of the bioactive compounds from papaya (*Carica papaya* Linn) leaves. A full factorial design with independent variables *viz*. Temperature (50, 60, 70 and 80 ºC) and time (3,6,9,12 and 15 min) were taken to analyze their effect on total phenolic content, total flavonoid content, % radical scavenging activity and total soluble solids of papaya leaf samples. The best conditions were observed at 50 ºC and 3 min. Among different treatment combinations, the maximum value of bioactive compounds is obtained at low temperature and time *viz*., 50 ºC and 3 minutes whereas minimum value is found at high temperature and time 80 ºC and 15 minutes. In blanching, high temperature and time led to the reduction of bioactive compounds in papaya leaves.

Keywords: Papaya leaves, blanching, phenolic content, flavonoid content, %RSA, and chlorophyll

1. Introduction
Carica papaya L. is a large perennial tree and herbaceous plant from the Caricaceae family and it is thought to be native to the tropical America. This papaya fruit commonly called as paw paw, is one of the main products from the papaya plant (Ayoola & Adeyeye, 2010; Vuong *et al*., 2013) [1, 11] while remaining portions of the plant like leaves, stems and trunks are considered as by-products. Generally, the papaya plants can grow in between 5–10 m height. These leaves having a diameter of 50–70 cm, are profoundly with 7 lobes. In many regions of the world, they had been utilised as a remedy for a variety of illnesses (Vuong *et al*., 2013 and Raja *et al*., 2019) [11, 9]. Compared to other therapeutic plants, papaya leaves have a good proportion of tannins, terpenoids, and phenolic acids. (Fasola and Iyamah, 2014; Raja *et al*. 2019) [5, 9]. Bioactive compounds namely phenols, flavonoids, alkaloids, saponins, cardiac glycosides, tannins and steroids were noticed in the phyto-chemical screening of papaya leaves extract (Fadzilah *et al*., 2020) [6]. The phytochemicals, vitamins, and mineral composition of green papaya leaves were higher compared to that of brown and yellow papaya leaves (Ayoola and Adeyeye, 2010) [1]. These leaves also had more crude protein, carbohydrate, crude fibre, Ca, Mg, Fe, and K compared to that of papaya fruit pulps and seeds (Nwofia *et al*., 2012) [8]. The papaya leaf extract is enriched with phenols, vitamins and proteolytic enzymes which acts as a good source of antimicrobial and antioxidant agent (Maisarah *et al*., 2013; Banala *et al*., 2015) [7, 3]. Plant phenolic compounds, and their secondary metabolites– flavonoids and proanthocyanidins, have been frequently reported as the active bioactive components associated with antioxidant properties and health benefits. It is well known that polyphenols are susceptible to oxidative degradation, particularly during lengthy exposure to heat, light, and oxygen; thus the different methods like blanching can significantly affect the stability of polyphenols (Vuong *et al*., 2013) [11].
Blanching is the process of mild heat treatment given to fruits and vegetables followed by cooling before any processing operation. This helps to inactivate enzymes, modifies texture while maintaining nutritional value in food product. This blanching process also helps in eliminating air that could have been trapped in the cells of fruits. It is usually carried out in hot water or in steam. It also helps to reduce or eliminate the bitterness of the vegetables and acid components that are common in leaves (Bamidele *et al*., 2017) [4].
There had been much information available on the effect of blanching on the nutritional composition of fruits and vegetables; however, little information is available on the effect of blanching in papaya leaves.

The main aim of this study was to examine the effects of blanching parameters on the stability of bioactive compounds from papaya leaves.

2 Materials and Methods

2.1 Sample preparation

The mature fresh papaya leaves were sourced from the Precision Farm Development Centre (PFDC) of Central Institute of Agricultural Engineering, Bhopal. The healthy papaya leaves were sorted manually and stored at 4 ºC prior to experimentations to minimize deterioration of bioactive compounds. These fresh mature leaves were washed with distilled water.

2.2 Blanching of papaya leaf

Fresh papaya leaves were taken and the veins of leaves were separated manually. These papaya leaf samples were chopped into small pieces by using knife. About 20 g of papaya leaves were taken and kept in a 400 ml of distilled water in a beaker. This sample is positioned in a temperature controlled water bath. At different temperatures of 50 ºC, 60 ºC, 70 ºC and 80 ºC with different time levels *viz*., 3 min, 6 min, 9 min, 12 min and 15 min, Papaya leaves were blanched. After arriving setting time, leaves were taken out from hot water and submerged in cool distilled water for 2 minutes. Water from leaves was removed after cooling, and then these papaya leaf samples were used for further biochemical analysis.

2.3 Analysis of papaya leaves

All the samples which were treated by blanching method were analysed for total phenolic content, % Radical scavenging activity, total flavonoid content and total soluble solids

2.3.1 Total phenolic content

The Folin & Ciocalteu phenol reagent was used to quantify total phenols using a spectrophotometric method (Singleton and Rossi, 1999) [10]. 1 ml of papaya leaf extract was combined with 9 ml of distilled water, and then 1 ml of Folin & Ciocalteu's phenol reagent was added. After 5 min duration, 10 ml of 7% sodium carbonate (Na_2CO_3) solution was mixed to it properly and the volume was made to 25 ml by using distilled water. A vortex shaker was used to properly combine this solution (VS-1108, Sunline). After incubation time of 90 minutes, the absorbance of samples was measured at a wavelength of 750 nm using spectrophotometer (A116352, Shimadzu corp., Japan). The total phenolic content was measured by the gallic acid standard with the help of calibration curve. The results were obtained in mg of gallic acid equivalent/g with the below equation:

$$\text{TPC}\left(^{\text{mg GAE}}/_{\text{g}}\right) = C_p \times \frac{V}{M}$$

Where, C_p- gallic acid concentration, mg/ml
V-volume of extract, ml and M- sample weight, g

2.3.2 Total flavonoid content

The total flavonoids in papaya leaves were measured using a spectrophotometric method using an aluminium chloride process (Zhishen *et al*., 1999) [12]. 1 ml of papaya leaf extract is added to 4 ml of distilled water. Then 0.3 ml of sodium nitrite at 5% (w/v) concentration was combined and the mixture was allowed to stable for a duration of 5 min. After that, 0.3 ml of aluminium chloride ($AlCl_3$) was added at 10% (w/v) concentration and allowed to stable for 6 min. It was then mixed with 2 ml of 1M sodium hydroxide (NaOH) and added distilled water to made a volume of 10 ml. The absorbance of samples was measured at a wavelength of 510 nm using spectrophotometer (A116352, Shimadzu corp., Japan). The total flavonoid content was measured by the quercetin standard with the help of calibration curve. The results were obtained in mg of quercetin equivalent/g with the below equation:

$$\text{TFC}\left(^{\text{QE}}/_{\text{g}}\right) = C_f \times \frac{V}{M}$$

Where, C_f- quercetin concentration, mg/ml
V-volume of extract, ml and M- sample weight, g

2.3.3 % radical scavenging activity by DPPH

Brand-Williams *et al*., (1995) [2] was adapted for the determination of % radical scavenging activity. A DPPH solution was made by adding of 3.94 mg of DPPH in 99% methanol of 100 ml. 1 ml of papaya leaf extract was mixed with 3 ml of DPPH solution. This mixture sample was allowed to incubate for 20 minutes duration in dark condition. The absorbance readings of the papaya leaf samples and control were measured at a wavelength of 517 nm by spectrophotometer. The % radical scavenging activity by DPPH was measured with the below equation:

$$\%\text{RSA} = \left(\frac{A_C - A_S}{A_C}\right) * 100$$

Where, A_c - absorbance of control sample
A_s- absorbance of papaya leaf sample.

2.3.4 Total soluble solids

A digital pocket refractometer (Atago, Japan) was used for the measurement of TSS in ºBrix.

2.4 Statistical analysis

The study was carried out by using full factorial design as experimental plan. All the experiments were carried out in triplicates. This results were analyzed through the analysis of variance (ANOVA) at $P<0.05$. To perform the statistical analysis, Design expert software (Stat-Ease®, Trial-Version 12, Minneapolis was utilised.

3. Results and Discussion

3.1 Total phenolic content (TPC)

The experimental results for the total phenol content of blanched leaf samples at different temperatures *viz*., 50, 60, 70, and 80 ºC and time duration *viz*., 3, 6, 9, 12, and 15 min was shown in Fig.1.The value of TPC in control sample is 201.01±3.48 mg GAE/g whereas TPC in blanched leaves were found between 73.43±1.18 to 187.5±5.42 mg GAE/g. The values of TPC in blanched papaya leaf samples were lower than that of control samples. The effect of blanching on total phenolic content was found significant ($P<0.05$) with $R^2=0.99$. It was observed that TPC was decreased with the increase of temperature. Similar pattern was observed with blanching time. TPC was decreased with the rise of blanching duration from 3 to 15 min. Among various treatment combinations, the highest value of TPC was found at low temperature and time *viz*., 50 ºC and 3 min, whereas lowest value was found at high temperature and time *viz*., 80 ºC and 15 min. High temperature and time attributed to the loss of

total phenolic content in blanching of papaya leaves. This might be due to the dilution effect of phenolic compounds in water that causes significant losses in phenol compounds during process of blanching.

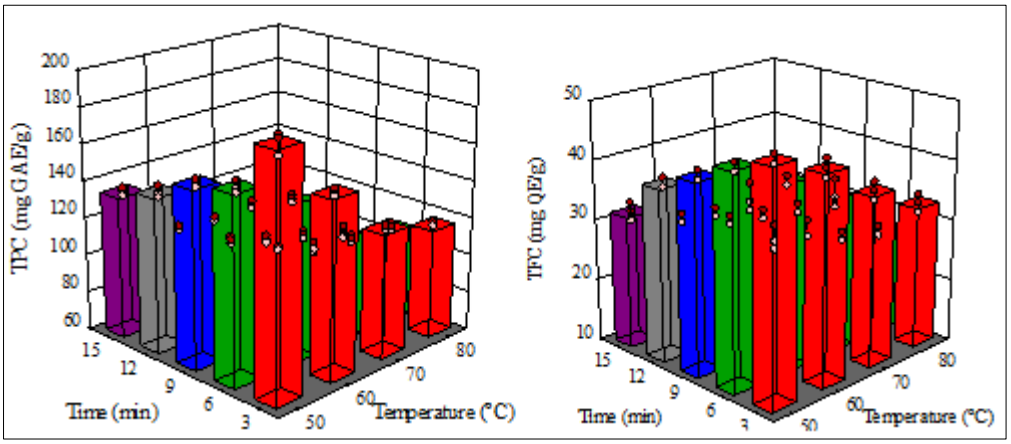

Fig 1: Effect of blanching parameters on TPC and TFC

3.2 Total flavonoid content

ANOVA test was done for total flavonoid content (TFC), and it was observed significant at $P<0.05$ with high coefficient of determination, $R^2=0.98$. Blanching factors namely temperature, time as well as its interaction shown significant effect ($P<0.05$) on TFC. The value of TFC in the control sample is 53.76±0.7 mg QE/g F.W. TFC values in blanched leaf samples were lower than that of control values. The values of TFC were found between 18.88±2.3 to 47.49±0.87 mg QE/g F.W. (Fig.1). With respect to temperature, it was noticed that TFC reduced as the rise of temperature. Blanching time also showed same tendency. With the rise of blanching duration *viz*., 3 to 15 min, TFC was decreased. The highest value of TFC was noted at low temperature and time i.e., 50 ºC and 3 min, whilst the lowest value was noted at high temperature and time i.e., 80 ºC and 15 min. Interaction of high temperature and time attributed to the reduction in total flavonoid content.

3.3 % radical scavenging activity (%RSA)

% radical scavenging activity (%RSA) was tested using ANOVA, and the results obtained were significant at $P<0.05$ ($R^2=0.97$). In this condition, temperature, time as well as the interaction of both parameters exhibited significant effect ($P<0.05$). The value of %RSA in control sample is 73.22±1.22%. The obtained %RSA in blanched samples was ranged between 50.1±1.22percent to 72.31±0.7%. Blanched leaf samples possessed lower values compared to that of unblanched leaf samples. Regarding blanching temperature, it was noticed that %RSA was declined with the rise of temperature. In blanching time, %RSA was decreased with the rise of blanching time from 3 min to 15 min (Fig. 2). The lowest and highest value of %RSA was found at 80 ºC, 15 min and 50 ºC, 3 min, respectively. %RSA follows similar pattern as TPC and TFC of blanched papaya leaves. The blanching process might increase the solubility and leaching in the antioxidant phytochemicals.

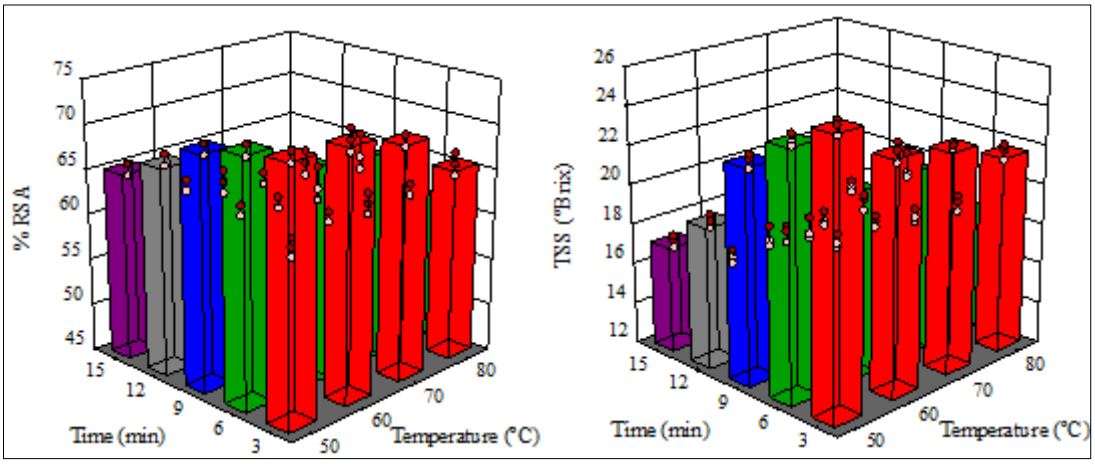

Fig 2: Effect of blanching parameters on %RSA and TSS

+3.4Total soluble solids (TSS)

Significant effect of blanching parameters was observed (R^2 = 0.98) on total soluble solids (TSS) of papaya leaves at $P<0.05$. ANOVA test revealed that single factors in blanching namely temperature, time as well as its interaction perceived significant effect on TSS. In the case of temperature, decrease on TSS was observed from 50 ºC to 80 ºC while in the case of blanching time, TSS reduced with the rise in blanching from 3 min to 15 min. Results shown that the maximum and minimum content of TSS was recorded at 50 ºC, 3 min and 80 ºC,15 min, respectively. TSS content reduced with the rise of temperature and time (Fig. 2). Control value in TSS was 25.96±0.36 ºBrix. It is also noticed that TSS values were higher in unblanched leaves compared to that of blanched leaves.

4. Conclusions

Blanching parameters like temperature and time significantly affects bioactive compounds in leaves. It concludes that high blanching temperature and time led to the reduction in bioactive compounds in papaya leaf extract compared to low blanching temperature. Low blanching temperature helps to retain bioactive compounds. Thus, selection of blanching temperature and time is most important parameter for retention of its bioactive compounds.

References

1. Ayoola PB, Adeyeye A. Phytochemical and nutrient evaluation of *Carica papaya* (pawpaw) leaves. Ijrras. 2010;5(3):325-328.
2. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. Lebensm. – Wiss. Technol. 1995;28:25-30. https://doi.org/10.1016/S0023-6438(95)80008-5
3. Banala RR, Nagati VB, Karnati PR. Green synthesis and characterization of *Carica papaya* leaf extract coated silver nanoparticles through X-ray diffraction, electron microscopy and evaluation of bactericidal properties. Saudi journal of biological sciences. 2015;22(5):637-644. 21. https://doi.org/10.1016/j.sjbs.2015.01.007
4. Bamidele OP, Fasogbon MB, Adebowale OJ, Adeyanju AA. Effect of blanching time on total phenolic, antioxidant activities and mineral content of selected green leafy vegetables. Current Journal of Applied Science and Technology. 2017;24(4):1-8.
5. Fasola TR, Iyamah PC. Comparing the phytochemical composition of some plant parts commonly used in the treatment of malaria. International Journal of Pure and Applied Sciences and Technology. 2014;21(1):1. https://doi.org/10.1.1.1083.9529
6. Fadzilah MF, Zubairi SI, Abidin NZ, Kasim ZM, Lazim A. Physico-chemical and sensory acceptance of *Carica papaya* leaves extract edible O/W emulsion as prospective natural remedies. Arabian Journal of Chemistry. 2020;13(11):7829-7842. https://doi.org/10.1016/j.arabjc.2020.09.014
7. Maisarah AM, Amira BN, Asmah R, Fauziah O Antioxidant analysis of different parts of Carica papaya. International Food Research Journal. 2013;20(3):1043-1048.
8. Nwofia GE, Ojimelukwe P, Eji C. Chemical composition of leaves, fruit pulp and seeds in some *Carica papaya* (L) morphotypes. International Journal of Medicinal and Aromatic Plants. 2012;2(1):200-206.
9. Raja KS, Taip FS, Azmi MMZ, Shishir MRI. Effect of pre-treatment and different drying methods on the physicochemical properties of *Carica papaya* L. leaf powder. Journal of the Saudi Society of Agricultural Sciences. 2019;18(2):150-156. https://doi.org/10.1016/j.jssas.2017.04.001
10. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in enzymology. 1999;299:152-178. https://doi.org/10.1016/S0076-6879(99)99017-1
11. Vuong QV, Hirun S, Roach PD, Bowyer MC, Phillips PA, Scarlett CJ. Effect of extraction conditions on total phenolic compounds and antioxidant activities of *Carica papaya* leaf aqueous extracts. Journal of herbal medicine. 2013;3(3):104-111. https://doi.org/10.1016/j.hermed.2013.04.004
12. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry. 1999;64(4):555-559. https://doi.org/10.1016/S0308-8146