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Effect of extraction temperature and technique on phenolic compounds and antioxidant activity of *Tamarindus indica* seeds

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Abstract

Natural antioxidants from tamarind seed pose preservative effect in food and disease protective benefits in biological system. Extraction being the initial and crucial most step of antioxidant recovery is influenced by many conditions. In this study the effects of technique and temperature during solvent extraction of phenolic antioxidants from tamarind seed have been studied. The extraction techniques compared have been shaking and magnetic stirring. A binary solvent system of 50 % ethanol has been used and extraction has been carried out at varied temperatures (ranging 25 - 60° C). Total Polyphenol Content (TPC) and Total Antioxidant Activity (TAA) determined at these conditions have indicated magnetic stirring as the most convenient and exhaustive technique. While the maximum TPC has been extractable at 60° C, the highest TAA has been recorded at the extraction temperature of 40 ° C. Results have indicated that extracting temperature and technique significantly (P<0.05) influenced the antioxidant property and total phenolic compound recovery from tamarind seed.

Keywords: Antioxidant, Extraction, Magnetic stirring, Polyphenols, Tamarind seed.

Introduction

In biological system oxygen has an ambivalent role to play. Though being indispensible for the production of energy in the cellular system, it may pose some detrimental effects such as neuro-degeneration, ageing, and cancer in conditions of oxidative stress[1]. Oxidative stress has been first defined by Sies[2] as "a disturbance in the prooxidant to antioxidant balance in favour of the former, leading to potential damage" giving much importance to the role of antioxidants in diet therapy and disease pathology. Natural antioxidants have gained much importance over synthetic antioxidants in modern discussion of food science and technology due to the aspects of food safety, sustainability and green label they add to a food product[3]. Tamarind seed, a food processing waste of tamarind pulp industry[4] is a rich but underutilised source of natural antioxidants and are abundantly available especially in the tropical areas[5]. The fact that 40% of the mass of tamarind fruit is contributed by tamarind seed[6] indicates that a substantial amount of seed is discarded or degraded in different stages of processing chain (Figure-1). Since food wastes are being considered as a cheap and abundant source of natural antioxidant[7] and existent technologies facilitate their recycling within the food chain, the present study is designed to explore the antioxidant activity of tamarind seed as a function of extraction temperature and technique. Many efforts have been intensified directing towards the isolation, characterisation and extraction of antioxidant compounds from tamarind seed. Extraction is the initial and the most crucial step in the multi step process of recovery of bioactive components with the highest antioxidant activity of the extract. Solvent extraction is widely practised to recover phenolic antioxidant from plants[8] which is achieved by a complex interplay between various conditions such as extraction time[9], temperature[10], solid to liquid ratio[11], chemical nature of the compounds, the extraction technique used[12], and the occurrence of interfering substances[12]. Literature study[13-15] indicates that commonly employed extraction techniques have been predominantly aided by agitation. Similarly thermal treatment also affects the outcome of extraction process as well as the energy cost associated with extraction. Though a sizable literature exists on the effect of extraction temperature, no published results have been found on the effect of extraction technique for recovery of antioxidant from tamarind seed. Considering the simplicity, effectiveness and convenience of operation, the present study aims to investigate the effect of magnetic stirring and water bath shaking at different temperatures for the extraction of phenolic antioxidants from the seeds of *Tamarindus indica.*

Materials and methods

Collection and preparation of sample: Due to the profound availability of the seeds and almost zero transit charges to the work place, tamarind seeds have been collected from the local markets adjacent to Jadavpur University Campus. 2 Kg of sample has been procured from the fruit sellers at once and has been used throughout the study. The cleaned, sorted and graded whole seeds have been sun dried for 7 hours for 4 consecutive days at an average atmospheric temperature of about 39^0 C to remove the surface moisture and stirred occasionally to prevent mold infestation. Further, seeds have been oven dried at 70^0 C (Hot air oven; make: Sicco Instruments Pvt. Ltd., Calcutta, India) to a moisture content of 9 % and the particle size has been reduced to an average of 0.25 mm (ASTM sieve IS standard) by Denver Lab patented crusher grinder. The sample thus obtained has been stored in air tight high density polyethylene safety container and designated as TSP (Tamarind Seed Powder).

Chemicals and reagents: The chemicals which have been used for the purpose of investigation are Gallic Acid of S.d. fine-chem. Ltd Mumbai, India, 2,4,6-Tri(2-pyridyl)-S-triazine (TPTZ) of Himedia, Mumbai, India, Sodium Carbonate, Iron(III) chloride 6-hydrate, Iron(II) sulphate 7-hydrate, acetic acid, Sodium Acetate, Ethanol, Hexane, Hydrochloric Acid, Folin–Ciocalteu's Phenol of Merck (Germany). All analytical grade chemicals have been used and distilled water has been utilised for reagent preparation.

Extraction of sample: Hydro- alcoholic extracts of phenolic antioxidants from TSP have been prepared using thermal agitation technique in temperature controlled shaker water bath (manufactured by Sicco Instruments Pvt. Ltd., Calcutta, India) and temperature controlled magnetic stirrer with hot plate (make: Remi.). A binary solvent system of 50 % (v/v) aqueous ethanol has been used and the extraction temperature has been varied as 30°C, 40°C, 50°C and 60°C during both the extraction techniques. Material to liquid ratio has been fixed at 1:20 in all the experimental set ups. Thermo resistant borosilicate beakers have been employed and their radius and length has been kept constant for each experimental set- up.

Extraction in water bath: A batch solvent extraction set up has been configured; agitation by shaking and application of thermal treatment has essentially aided the process of diffusion of the extractives, namely the phenolic antioxidants into the solvent. Figure-2 represents the experimental set up arranged for the extraction. It shows the to and fro movement of the water tank assembly inside the water bath which has induced the shaking of the beakers loaded with solvent and material. The beakers have been sealed with grade M parafilm prior to shaking in order to prevent oxidative losses of the antioxidants. Shaking speed has been controlled at the rate of 60 strokes per minute during the 2 hours of extraction at 30°C, 40°C, 50°C and 60°C.

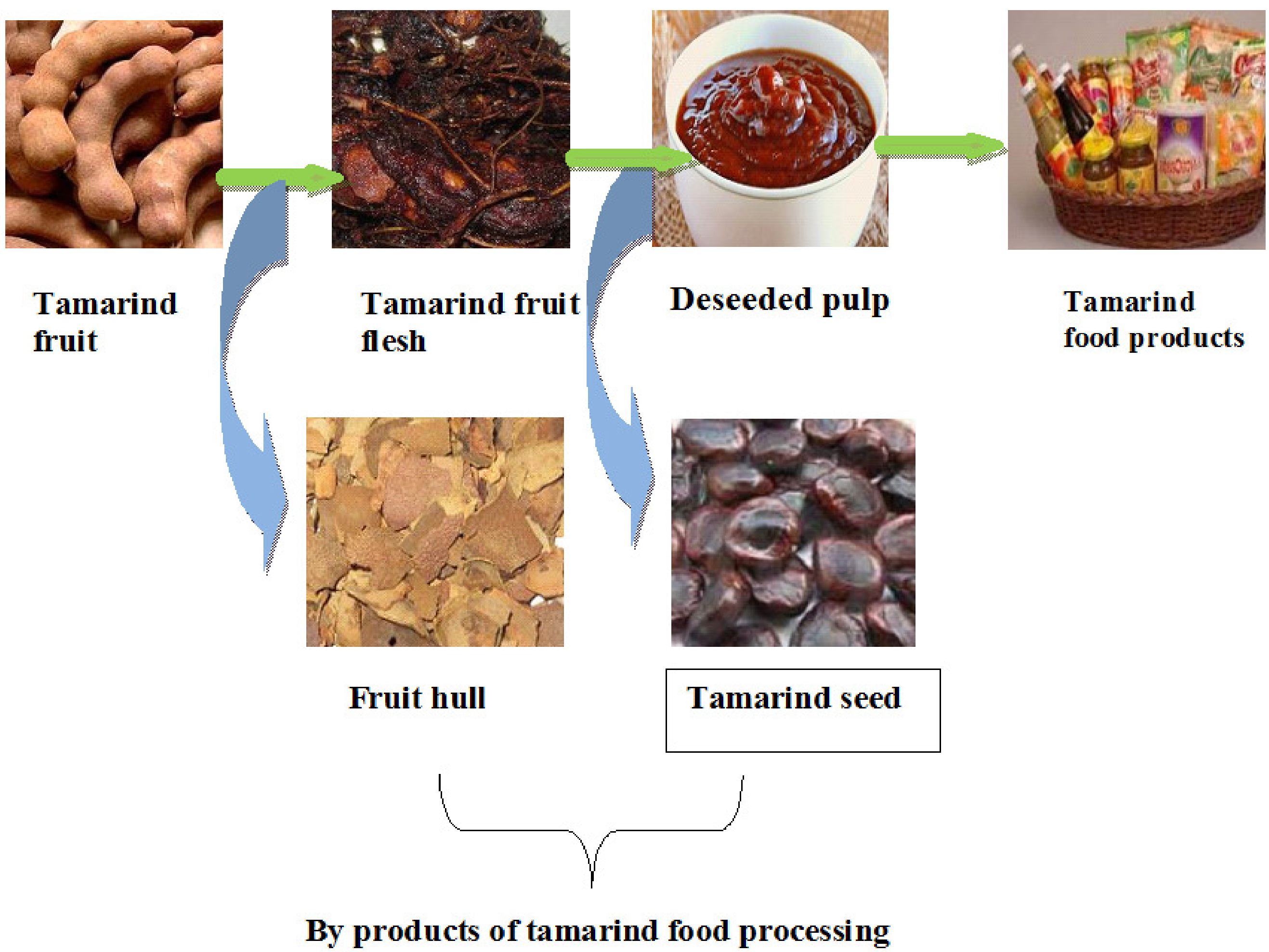

Figure-1: Tamarind seed – a by- product of tamarind food processing

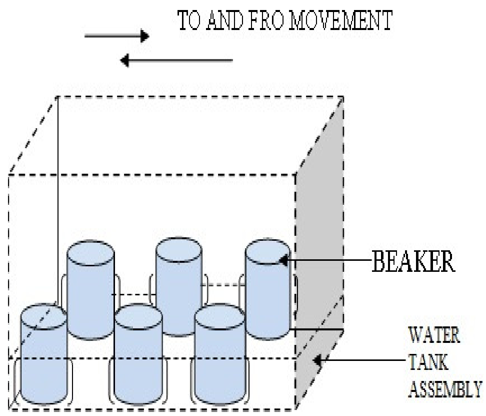

Figure-2: Extraction of phenolic antioxidants in shaker water bath.

Extraction in magnetic stirrer: This set up has essentially involved a rotating magnetic field to cause agitation by stirring the solution inside the extraction vessel. A 50 ml borosilicate thermo resistant beaker has been loaded with the sample and solvent mixture and placed over the hot plate. A teflon coated 3 cm long magnetic stir bar has been immersed into the extraction solution and the beaker has been sealed with grade M parafilm. The motion of the stir bar, in turn, has been driven by another rotating magnet in the stirrer device, beneath the extraction vessel (Figure-3). Rotation of the stir bar has been controlled by speed regulator unit and maintained at 100 rpm and the extraction temperatures have been regulated by temperature control unit at at 30°C, 40°C, 50°C and 60°C and extracted for 2 hours.

The subsequent steps which followed extraction have been defatting and filtration as described in the previous work[16] by the authors. The filtrates from magnetic stirring and water bath shaking have been designated as MSE (Magnetic Stirrer Extract) and WBE (Water Bath Extract) respectively which have been directly used for estimation of TPC and TAA. Duplicate runs have been taken for each extraction technique followed and all the analyses have been performed with 3 times reproducibility.

Determination of total polyphenolic content (TPC): TPC extracts has been determined by the method described by Malik and Singh, 1980[17] with some modifications. Briefly, 0.75 ml of different concentrations of the extracts have been mixed with 3 ml of distilled water and 0.5 ml of Folin- ciocalteu reagent (diluted to 1:1 with water) and 1 ml of 20% Na_2CO_3. After 120 minutes the absorbance has been read by spectrophotometer (Hitachi U-2000) at 760 nm and plotted in a standard calibration curve of Gallic Acid. These results have been expressed as mg Gallic Acid Equivalents per gram of dry sample.

Determination of total antioxidant content (FRAP assay): The FRAP assay has been carried out according to Benzie and Strain[18]. Briefly, the FRAP reagent has been prepared freshly from sodium acetate buffer (300 mM, pH 3.6), 10 mM TPTZ solution (dissolved in 40 mM HCl) and 20 mM iron(III) chloride solution in a ratio (v/v) of 10:1:1, respectively. It has been warmed to 37°C in a water bath before use and 3 ml of it has been added to 100 µl of the sample solution. The absorbance has been measured at 593 nm using spectrophotometer after 4 min and plotted in a standard calibration curve of $FeSO_4$ solution. The results have been expressed as µmol Fe (II)/g dry sample.

Statistical Analysis: Results have been expressed as mean ± standard deviation of triplicate assays and analysed by Microsoft Excel 2007 and. Significant levels of difference has been analysed in MINITAB (version 17) using paired t – test and defined using the values $p < 0.05$. Pearson correlation (r) and R^2 values have been utilised to express correlation estimations.

Results and discussion

The focus of the present study has been on the estimation of total polyphenol content (TPC) and total anti oxidant activity (TAA) of *T. indica* seed extracts obtained by shaking in water bath (WBE) and magnetic stirring (MSE) at different temperatures. The effectiveness of phenolic antioxidant extraction has been significantly higher in MSE than WBE. TAA (FRAP) and TPC at different extraction techniques and temperatures have been recorded and shown in Table-1. The results showed that TPC and TAA vary with the extraction techniques and extraction temperature. Maximum TPC and TAA have been obtained by magnetic stirring and recorded as 48.63 mg GAE and 217.12 µmol Fe (II) g^{-1} dry sample respectively. Our reports are in agreement with previous work[12] where greater extraction of phenolics from pink flesh guava has been achieved by magnetic stirring compared to shaking in water bath. Both the processes have utilised thermal agitation as the main principle of extraction, but the greater antioxidant activity of MSE may be due to the higher shear owing to higher stirring speed generated during magnetic stirring than during shaking in water bath. Magnetic stirring utilised the technology of mixing the constituents inside the vessel whereas, the entire vessel has been agitated in water bath shaking. During the process of extraction in magnetic stirrer molecular diffusion and chaotic advection force[19] have been generated by solid body rotation of the magnetic stir bars in the solution. While in shaker water bath the beakers have been shaken on vertical axis between the opposed arms by vigorous forward and backward movements of the arms to mix the solution thoroughly. The higher level of agitation in stirring directed towards more efficient molecular disruption compared to the effect of shaking. The higher the shear the more has been the increase in surface area conferring high surface to volume ratio that has facilitated the leaching of phenolics into the solvent[20]. This may be the reason of greater TPC and TAA recovery in MSE. Previous works[21-22] utilising magnetic stirring for extraction of phenolic compounds from plant materials have shown stirring speed to be crucial for greater yield.

Table-1: TPC and TAA (FRAP) in WBE and MSE at varying extraction temperatures.

	25˚C		30˚C		40˚C		50˚C		60˚C	
	TPC	FRAP	TPC	FRAP	TPC	FRAP	TPC	FRAP	TPC	FRAP
WBE	28.08± 1.15[a]	190± 1.92[b]	34.88± 1.58[a]	208± 2[a]	35.72± 3.53[a]	210.03± 1.93[b]	41± 2.62[a]	198.40± 2.26[b]	44.82± 1.39[a]	168.62 ± 1.46[a]
MSE	33.05± 1.93[b]	196± 2.22[a]	36.38± 1.46[a]	214± 2[a]	38± 2.06[a]	217.12± 2.12[a]	43.97± 1.26[a]	192.44± 2.11[a]	48.63± 0.84[b]	165± 2.96[a]

Data shown are the average and standard deviation based on triplicate runs (mean value ± standard deviation), Different letters in superscript in a column denote statistically significant ($p<0.05$) difference in the values.

The effect of extraction temperature on TPC and TAA has been investigated in the temperature range of 25 – 60°C. The results show a linear rise in TPC as extraction temperature increases with a maximum TPC of 48.63 mg GAE g^{-1} dry sample at 60˚C when extracted in magnetic stirrer. The relationship between extraction temperatures with TPC of WBE and MSE have been shown in Figure-4 and Figure-5. Strong positive correlation (R^2 =0.97 in WBE and R^2 = 0.93 in MSE) have been observed which may be attributed to the fact that solid to liquid extraction is led by the principle of diffusion which, in turn is influenced by extraction temperature[10]. The increase in TPC with application of heat may be due to the increased diffusion coefficients resulting in increased solubility of polyphenol in solvent[23]. Moreover, diffusion is the result of the random movement of the solutes into the solution. Increasing the extraction temperature has increased the kinetic energy of the moving particles causing more effective leaching. Further, literature review[24] suggests that Phenolic acids occur in plants as the intermediates of metabolic pathways, sequestered in the vacuoles or in cell wall component. Cellular constituent has been broken down by thermal processing which has released more phenolic acids from the bound state. However, the antioxidant activity has been found to decline with rise in temperature above 40°C. According to previous researchers[25] this may be due to thermal decomposition of other antioxidant compounds than polyphenols present in the sample. The higher shear of magnetic stirring adding to the effect of rise in temperature may be the reason of the greater decline in TAA above 40°C in MSE compared to that of WBE. The higher agitation of magnetic stirring may have caused disruption of cell wall leading to the release of more oxidative and hydrolytic enzymes eventually destroying more antioxidants[26] in MSE. However, further increase in extraction temperature have not been performed due to volatile nature of ethanol and its boiling temperature of 78.24 ± 0.09°C [27].

The correlation between TPC and TAA at different extraction temperatures has been estimated to comprehend the contribution of phenolic compounds in total antioxidant activity of TSP. A positive and linear correlation (r = 0.99) between TAA and TPC in the different extracts has been observed during rise in extraction temperature from 25- 40˚C; nevertheless a negative correlation (r = -0.45) between TPC and TAA has been observed at 50 and 60˚C. The result has been indicative of the implication that both in WBE and MSE polyphenols have been mostly responsible for antioxidant activity upto 40˚C but as temperature increased a simultaneous fall in TAA but rise in TPC occurred, indicating degradation of antioxidant components other than polyphenols at these higher temperatures.

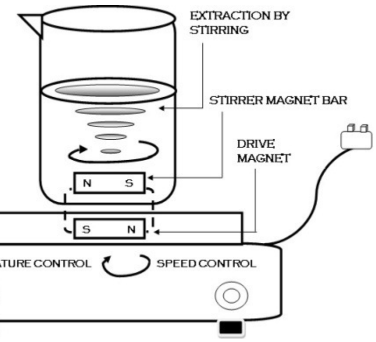

Figure-3: Extraction of phenolic antioxidants in magnetic stirrer with hot plate.

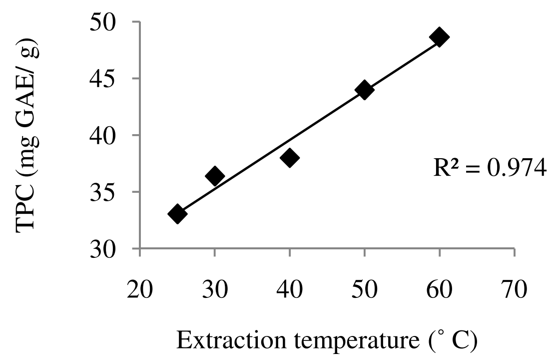

Figure-4: correlation analysis of TPC with extraction temperature in WBE.

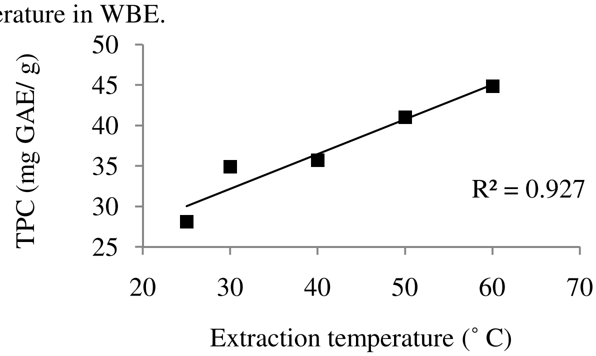

Figure-5: correlation analysis of TPC with Extraction Temperature in MSE.

Conclusion

This study indicated that seeds of *Tamarindus indica* have noteworthy antioxidant activities, which depends on the extraction technique and extraction temperature. Effectiveness of extraction has been found to be dependent on the solubility of antioxidant compounds in the extraction solvent influenced by the extraction temperature and technique used. In the present study batch extraction technique of magnetic stirring has been established to be a simple one step extraction technique giving better results when compared to shaking in water bath. Shaking speed has been found to be a contributing factor in extraction of phenolic antioxidant from TSP along with extraction temperature. Maximum TPC has been recorded at 60˚C whereas highest antioxidant activity has been achieved at 40˚C after which TAA declined. Hence taking into consideration the nutraceutical benefits of antioxidant over quantitative yield of total phenolics, 40°C has been concluded as the most favourable extraction temperature while magnetic stirring has been emerged as the suitable technique for phenolic antioxidant extraction from the seeds of *Tamarindus indica.*

Nomenclature: FRAP - Ferric ion Reducing Antioxidant Power, GAE - Gallic Acid Equivalent, GRAS - Generally Recognized As Safe, MSE - Magnetic Stirrer Extract, TAA - Total Antioxidant Activity, TPC - Total Polyphenol Content, TSP - Tamarind Seed Powder, WBE - Water Bath Extract.

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