Antioxidant Activities of Spray Dried Tamarind Pulp Powder as Affected by Carrier Type and their Addition Rate

Shridhar N. Bhusari, and Pradyuman Kumar

***Abstract*—** Spray dried tamarind pulp powder (TPP) was prepared by using three carrier agents maltodextrin (40, 50, 60%), gum arabic (40, 50, 60%) and whey protein concentrate (10, 20, 30%) and their total phenolic content and antioxidative properties (by DPPH, FRAP and ABTS assay). Total phenolic content of TPPs ranged from 59.45-131.33 mg of GAE / 100g. It was observed that phenolic content was protected at higher carrier agent addition rates. Values of Radical scavenging activity (% RSA), FRAP (mg of Ferrous sulphate equivalent / g) and total antioxidant activities (TAA) by ABTS assay of TPPs varied from 61.73 to 76.43, 56.81-311.63 and 0.071 - 0.15 mM of trolox equivalent / g of powder. FRAP values of TPPs ranged from and showed decrease in FRAP values with increase in the addition rate of the MD and GA. Antioxidant properties were positively correlated with total phenolic content of TPP.

***Keywords*—** antioxidative properties, gum arabic, maltodextrin, spray drying, tamarind pulp powder, whey protein concentrate

I. Introduction

ANTIOXIDANTS are useful for providing protection against oxidative damage. Some antioxidant compounds can be generated in the body; but in inadequate amounts to protect against the oxidative load. Hence, adequate amounts of antioxidants are important to prevent build up of free radicals and oxidative damage in the body. Plants are rich alternative sources of natural antioxidants which can complement the antioxidants produced by the human body [1]. Tamarind belongs to the dicotyledonous family Leguminosae [2] and cultivated mainly for the fruit pulp to prepare a beverage and to flavour confections, curries, and sauces and is accepted as herbal medicine in parts of the world [3].

Antioxidant activities of the various parts of *T. indica* are reported [1], showed the potential of this fruit as a source of phenolic antioxidants. Tamarind pulp is rich in several phytonutrients that act as powerful dietary antioxidants and total phenolic content and showed a strong correlation [4]. Tamarind flesh showed greater antioxidant potential and phenolics content as compared to flesh of avocado, jackfruit, longan and mango [5]. Tamarind fruit extracts has the potential to control the risk of atherosclerosis development in humans by improving the efficiency of the antioxidant defense system [6] and can be used as a laxative and in treatment of abdominal pain [7].

India is the major sour tamarind producer in Asia with annual production is about 0.202 m tonnes in the year of 2012-13 (Spice Board of India). Tamarind powder is an interesting product because of its properties such as a long shelf life due to low water activity, low logistic expenditures and easy to use [8].

In spray drying a liquid product is atomized in a hot gas current to obtain a powder instantaneously [9], [10] and offering great scope for production of healthy and nutrient rich dried products [11]. Stickiness, hygroscopicity and solubility are some of the inherent problems of spray dried fruit juice powders [12] and results in lower yield and operational problems. To reduce the stickiness problem, available approaches include process based (such as use of low temperature and low humidity air) and material science based methods (use of drying aids) [13]. Addition of carrier agents, like polymers and gums to the product atomization can resolve these problems. Maltodextrins and gum arabic are the common carrier agents used for fruit juice drying [10] which influences physico-chemical properties and stability of the powder [14].

Spray drying has been widely used in the microencapsulation of food ingredients susceptible to deterioration by external agents and consists of entrapping an active agent (solid particles, liquid droplets or gaseous compounds) in a polymeric matrix, in order to protect it from adverse conditions. The immediate drying of the mixture leads to the formation of a matrix system in which the polymer forms a tridimensional network which contains the encapsulated material [15]. Bayberry powder was successfully obtained when the juice was spray dried with maltodextrin as the carrier. The retentions of total phenolic content and anthocyananin during drying process were about 96 and 94% and suggested that spray drying was a satisfactory technique for drying heat sensitive polyphenols [16].

The aim of this work was to evaluate the influence of carrier type i.e. maltodextrin, gum Arabic and whey protein concentrate and their addition rate on the total phenolic

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content and antioxidative properties of a tamarind pulp powder (TPP).

II. Material and methods

Tamarind (*Tamarindus indica* L.) fruits were purchased from local market in Longowal, India. Tamarind pods of fully matured state were used for the pulp extraction. At first fruits were washed and skins, veins and outer coverings were manually removed. Then fruits were washed and soaked in cold water with 1:1 proportion of water and tamarind fruits on weight basis overnight. Then whole mass was heated to 60 ^{o}C and pulp was extracted manually using muslin cloth. Remaining mass was again heated with small addition of water and again strained through muslin cloth and stored at 4 ^{o}C until use. Different proportions of tamarind pulp (based on TS) and carrier agent (on dry basis) were prepared. Proportions of 40, 50 and 60% for maltodextrin (MD 20 DE, Himedia lab, India) and gum arabic (GA) (Loba chemicals, India) and 10, 20 and 30% for whey protein concentrate (WPC) (Mahaan Proteins, India), by weight, were chosen on the basis of trials and in accordance with references for spray drying. Before dehydration, the final feed mixture was diluted and standardized with distilled water to 20°Brix TSS.

A. Spray drying

Powder was produced with pilot plant Spray-dryer (S.M. Scientech, India) with a cocurrent air flow. The speed of blower was set at 2000 rpm for all the drying. Distilled water was pumped into the dryer at a set flow rate of 18 ml/min to achieve the inlet/outlet temperatures of 180°C and 80°C, respectively. The dryer was run at this condition for about 10 min before the feed was introduced. Then feed mixture was introduced in spray dryer at already set conditions. The product was collected in a pre-weighed, insulated glass bottle connected at the end of cyclone collector and then packed in polyethylene bags. The bags were then stored in a desiccator containing silica gel before analysis.

B. Sample preparation of tamarind pulp powder (TPP) for antioxidant tests

50 mg of each powdered samples were weighed and dissolved in 1 ml of millipore water each. Then samples were sonicated for 5 min, subsequently they were kept in water bath at 60 ^{o}C for 20 min and again samples were sonicated and centrifuged at 10000 rpm. Supernatants were taken for estimation.

C. Total Phenolic Content (TPC)

Total phenolic content was determined by using Folin-Ciocalteau reagent method [17]. 100 µl of the TPP sample extract was mixed with 5 ml of distilled water in a test tube, followed by addition of 500 µl of Folin-Ciocalteau reagent. After 30 s and before 8 min, 1.5 ml of sodium carbonate (20% w/v) was added. Then mixture was kept in water bath for 30 min at 40°C and the absorbance was measured at 765 nm with UV-VIS spectrophotometer (Jasco-V-530, Japan). The total phenolic content in the sample was then calculated by using standard calibration curve of Gallic acid. The total phenolic content was expressed as mg of Gallic acid equivalent (GAE)/100g of TPP.

D.% Radical scavenging activity by (DPPH) antioxidant assay

Procedure described by Moon and Shibamoto [18] was used with slight modifications. The principle of DPPH assay involves reaction of the antioxidants with the stable DPPH radical, converting the complex from a deep violet color to a colorless complex. The degree of discoloration indicates the scavenging potentials of the samples [1].

100 µl of TPP extract was taken and then 100 µl of 1 mg/ml of DPPH solution was added to this extract. Then it was diluted to 3 ml by methanol, incubated at room temperature for 30 min and absorbance was measured at 517nm with UV-VIS spectrophotometer (Jasco-V-530, Japan). The absorbance was taken as sample absorbance. For the control 100µl of DPPH solution was diluted to 3 ml and absorbance was taken at 517 nm and % radical scavenging (% RSA) activity was calculated as follows

$$\% \text{ RSA} = ((\text{Absorbance of control} - \text{Absorbance of sample})/ (\text{Absorbance of sample})) \times 100$$

E. Ferric reducing ability of plasma (FRAP) assay

The ferric reducing ability of plasma (FRAP) assay uses antioxidants as reductants in a redox-linked colorimetric reaction, reducing a ferric-tripyridyltriazine (Fe (III)-TPTZ) complex to the ferrous, Fe (II) form [19], forming an intense blue colour complex which can be measured colorimetrically. Acetate buffer (300 mM, pH 3.6), 10 mM TPTZ solution in 40 mM HCL and 20 mM $FeCl_3.6H_2O$ were prepared and mixed reagents in the ratio 10:1:1 respectively. Sample (100 µl) extract is mixed with 5ml of working FRAP reagent and absorbance is measured at 593nm just after vortexing. Thereafter, samples were placed at 37 °C in water bath and absorbance is again measured after 4 min at 593 nm using UV-VIS spectrophotometer (Jasco-V-530, Japan).. The differences between the two absorbance readings were measured and compared to the calibration curve. $FeSO_4$ was used to prepare calibration curve and FRAP values were expressed as mg ferrous sulphate equivalents per g of TPP.

F. Total antioxidant activity by (ABTS) radical cation assay.

The total antioxidant activity spray dried TPP extracts was measured by the $ABTS^+$ radical cation decolorization assay involving preformed $ABTS^+$ radical cation. This assay is based on the inhibition, by antioxidants, of the absorbance of the radical cation of 2,2′-azinobis (3-ethylbenzothiazoline 6-sulphonate) ($ABTS^+$) at a wavelength of 734 nm [20]. 7 mmol ABTS was dissolved in distilled water and ABTS radical cation ($ABTS^+$) was produced by reacting ABTS stock solution with 2.45 mmol potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12‑16 h before use followed by adjustment of the absorbance of the

reactant to 0.700 ± 0.02. 50 µl of extract and 5 ml of $ABTS^+$ solution was taken in volumetric flask and incubated for 6 - 8 min in dark and absorbance was taken at 734 nm with UV-VIS spectrophotometer (Jasco-V-530, Japan).. Trolox was used as the standard and a calibration curve was plotted. Total antioxidant activity was calculated by plotting corresponding % Inhibition of sample in standard curve and was expressed in terms of Trolox equivalents (mM) / g of TPP.

G. Statistical analysis

All tests were carried out in at least three replications and the results are reported as the means with standard deviation. The data for the antioxidative properties were analyzed using ANOVA. The factors (and levels) included in the models were carrier agent type (maltodextrin, gum arabic and whey protein concentrate) and addition rate. The models included the main effects of carrier agent and addition rate and their interaction. Differences between means at the 5% and 1% level were considered as significant and highly significant respectively.

III. Result and Discussions

A. Total phenolics content (TPC)

Phenolic compounds are secondary plant metabolites with beneficial biological effects and most important is their documented action as potent antioxidants. Hence, it is common practice to measure both phenolic content and antioxidant activities when investigating the antioxidant potential of natural products [1].

Total phenolic content of TPPs ranged from 59.45 - 131.33 mg of GAE/100g of TPP. However Soong and Barlow [5] reported that total phenolic content of the tamarind flesh was 160 mg of GAE/100 g. So these results showed that during drying total phenolic content was reduced. But this reduced fraction may be due to the increasing fraction of the carrier agents.

It can be observed from Fig.1 that retention of phenolic content was increased with increase in carrier agent addition rate which indicates that total phenolic contents were well encapsulated at higher carrier agent addition rates. Same trend of preservation of phenolic compounds was reported with higher addition of carrier agents and suggested that the increased total phenolic content might be due to interference of carrier agent with the phenolic compounds [21]. It was also observed that TPPs with MD and GA does not show much difference when compared at same addition rate while the retention of phenolic compounds was highest in case of TPPs with WPC. Statistical data (Table I) showed that the effects of carrier agent and the addition rate of carrier agent on the total phenolic content was highly significant (P<0.01) while interaction effects between carrier agent and addition rate were non-significant.

B. % Radical scavenging activity by (DPPH) antioxidant assay

% Radical scavenging activity (% RSA) ranged from 61.73 - 76.43 (Fig. 2) and was increasing with increase in addition rate of carrier agents. This increasing trend was observed for all carrier agents. Similar trend was observed with spray-dried purple sweet potato flours [22]. But there was no significant difference in % radical scavenging activity for TPPs with all carrier agents when compared at same addition rate. Acai juice powders produced with maltodextrin and gum arabic did not significantly differ between each other with DPPH scavenging capacity after the spray drying process which is in agreement with our findings [15].

Statistical data (Table I) showed that the effects of carrier agent, addition rate of carrier agent and their interaction effects on the % radical scavenging activity were highly significant (P<0.01).

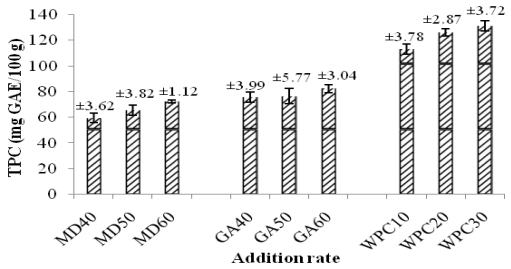

Fig.1 Effect of carrier type and addition rate on TPC of TPP

Table I
MEAN SUM OF SQUARE VALUES OF ANTIOXIDATIVE PROPERTIES OF TPP

Source of variation	TPC	% RSA	FRAP	TAA
Carrier agent	8313.59**	221.36**	78313.13**	0.009464**
Addition Rate	356.83**	24.038**	1747.88**	0.000857**
Carrier agent * Level	34.36	9.017**	4934.38**	0.000123
Error	13.76	0.262	13.44	0.000051

** P < 0.01 highly significant, * P < 0.05 significant

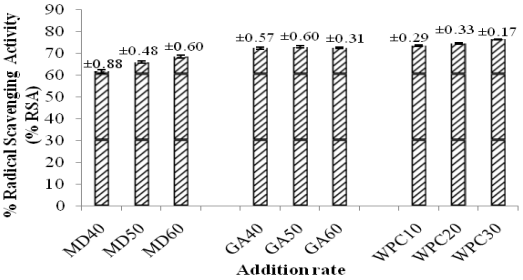

Fig. 2 Effect of carrier type and addition rate on % RSA of TPP

C. Ferric reducing ability of plasma (FRAP) assay

Antioxidants can be explained as reductants, and inactivation of oxidants by reductants can be described as redox reactions in which one reaction species (oxidant) is reduced at the expense of the oxidation of another antioxidant. The FRAP assay measures the antioxidant effect of any substance in the reaction medium as reducing ability [3].

FRAP values of spray dried TPPs were ranged from 56.81 - 311.63 mg of FSE/ g of TPP. It was observed that, the FRAP values of the TPPs were decreasing with increase in the

addition rate of the MD and GA and did not follow any trend with WPC.

It can be observed from Fig. 3 that TPPs with WPC showed higher FRAP values at same addition rate of MD and GA. These results were consistent with the results obtained in the other antioxidant assays i.e. TPPs with WPC showed higher antioxidant activities than TPPs with MD and GA. Statistical data (Table I) showed that the effect of carrier agent, addition rate of carrier agent with their interaction effects on ferric reducing antioxidant power was highly significant (P<0.01).

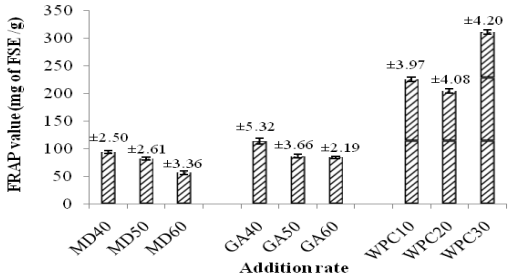

Fig. 3 Effect of carrier type and addition rate on FRAP value of TPP

D. Total antioxidant activity by (ABTS) radical cation assay.

The total antioxidant activities (TAA) of TPP were ranged from 0.071 - 0.15 mM trolox equivalent / g of TPP. It can also be seen from Fig. 4 that total antioxidant activity was increasing with increasing addition rate of carrier agents. This increasing trend was observed for all carrier agents. But there was no significant difference in TAA for TPPs with 50 and 60 % addition of MD. Similar results were observed with spray dried gac powder [23].

It can also be observed from Fig. 4 that there was not much difference between TAA of TPPs with MD and GA. Similar trends were observed with spray-dried acai juice powder [15]. It was observed that TPPs with WPC possessed higher antioxidant activity than TPPs with MD and GA. This difference might be attributed to the structural differences between carrier gents. Statistical data (Table 1) showed that the effect of carrier agent and addition rate of carrier agent on total antioxidant activity was highly significant (P<0.01) while the interaction effects between carrier agent and addition rate were non-significant.

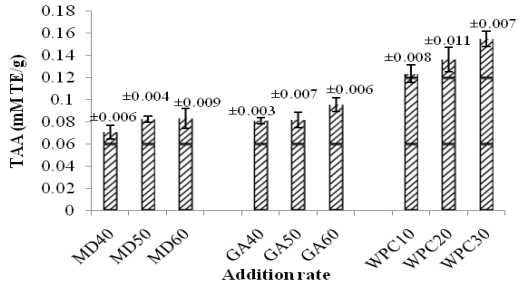

Fig. 4 Effect of carrier type and addition rate on TAA of TPP

E. Correlation between antioxidant activity and total phenolics content of the TPP

Correlation analyses between phenolic content and other antioxidant assays of TPPs were calculated using regression analyses. Results showed positive correlations between phenolic content and the total antioxidant activities by ABTS assay (r^2 = 0.9690) and in terms of FRAP values (r^2 = 0.847) so this showed that total phenolic content of TPPs mainly contributed to the antioxidant activity of the TPPs. Lower but positive correlation was found between % radical scavenging activity and total phenolic content of TPPs (r^2 = 0.640). Same trends were reported for antioxidant activity with phenolic contents in tamarind leaves, seeds and skins [1].

IV. CONCLUSIONS

The retention of antioxidants during drying process depends on the structure of the carrier agent as well as its addition rate. Antioxidant compounds were protected at higher carrier agent addition rates. Higher protection of the antioxidant was found for TPPs with WPC and same trend in all assays give its confirmation. The total antioxidant activities (TAA) of TPP with WPC were ranged from 0.12 - 0.15 mM trolox equivalent / g of TPP which was higher as compared to TPPs with MD and GA. This confirms the potential of protein for its use as carrier agent during spray drying. Positive correlations between phenolic content and the total antioxidant activities by ABTS assay (r^2 = 0.9690). These strong correlations between total phenolic content and other antioxidant assays also confirm strong antioxidant potential of phenolic compounds.

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