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ORIGINAL PAPER

Application of Tamarind Waste Extracts to Improve the Antioxidant Properties of Tamarind Nectars

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Abstract

Tamarind fruits are consumed worldwide and their seeds have an underexploited potential. We assessed the effect of the addition of a freeze-dried aqueous of extract tamarind seed (FAE) at three concentration levels (0.3, 1.15 and 2%) on the antioxidant capacity (DPPH, ABTS, FRAP and ORAC) and concentrations of total phenolic compounds in tamarind pulp. Conditions used to prepare the aqueous extracts were established using multivariate optimization. Moreover, nectars prepared from pulps combined with FAE were subjected to sensory tests. Tamarind fruits from three geographic regions in Brazil (Minas Gerais, São Paulo and Bahia) that were harvested in 2013 and 2014 were used in the study. Generally, the freeze-dried aqueous extracts increased the concentrations of antioxidants in the pulp. The results revealed a positive correlation between the FAE concentration in the pulp and the antioxidant capacity of all samples, particularly samples from Bahia and Minas Gerais, which presented an increase of up to 1,942% in the ABTS method when 2% FAE was incorporated into the pulp, from approximately 40.1 to 209.1 mM_{Trolox}/g_{dw} and 13.4 to 143.4 mM_{Trolox}/g_{dw}, respectively. Sensory tests indicated the satisfactory acceptance and non-distinction between nectar samples to which FAE was or was not added when the FAE concentration was less than $2.3g_{FAE}/L$, regardless of the geographic origin of the samples.

Keywords Aqueous extraction · Antioxidant capacity · Functional beverage · Juice · PCA (principal component analysis) · Sensory analysis · *Tamarindus indica* L.

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Abbreviations

FAE	Freeze-dried tamarind seed aqueous extract
DPPH	2,2-diphenyl-1-picrylhydrazyl; ABTS [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)]
FRAP	Ferric reducing antioxidant power
ORAC	Oxygen radical absorbance capacity
DL	Low density lipoprotein
TPC	Total phenolic compounds
CCD	Central composite design
ANOVA	Analysis of variance
S/L	Solid-to-liquid ratio
BA	Bahia state
MG	Minas Gerais state
SP	São Paulo state

Introduction

Fruits have been recognized for their benefits to human health. They contain high concentrations of non-nutritive and bioactive compounds, such as flavonoids, phenolic compounds, anthocyanins, and phenolic acids, as well as nutritive compounds, such as sugars, essential oils, carotenoids, vitamins, and minerals [1–3]. Tropical fruits are also increasingly recognized as presenting these functional properties in a vast biodiversity of cultivars with various structures and physiological characteristics [4–6].

Tamarind (*Tamarindus indica*), which is native to Africa and India, is produced in tropical and subtropical regions in the world and is widely incorporated in the diet of the local people [7]. Because of appropriate climate conditions in Brazil, tamarind fruits are currently produced all across the country. Typically, tamarind is consumed as a fresh fruit or used to produce beverages, desserts, ice creams and sauces [8].

Tamarind seed comprises 40% of the fruit weight and constitutes a by-product of tamarind processing, which is usually discarded by the industry and consumers. However, the seeds possess a high antioxidant potential, which has been attributed to specific compounds isolated from the seeds [9, 10]. These substances have been suggested to be associated with the *in vitro* pharmacological properties of aqueous extracts of tamarind seed. Moreover, *in vivo* studies using rats reported that these extracts may reduce the concentrations of triglycerides and total and LDL cholesterol, and contribute to controlling *diabetes mellitus* [11, 12].

Based on this information, the incorporation of tamarind seeds or extracts into food formulations is a feasible alternative to attain the benefits of their potential bioactivities. In this context, a determination of the antioxidant capacity of aqueous extracts of tamarind seeds is an important measure to support its use in the food industry to increase the functional properties of food products. Antioxidant capacity is typically measured using *in vivo* assays or chemically using *in vitro* assays, in which the capacity of extracts or specific compounds to neutralize chemical or radical species by either donating a hydrogen atom or transferring an electron is assessed [13, 14]. Because *in vivo* assays are costly and time-consuming, *in vitro* methods are widely used [13]. Antioxidant compounds may function through various mechanisms, and thus different assays of the antioxidant capacity have been used to study the antioxidant potential of vegetable extracts [15].

Currently, consumers are increasingly demanding food with health-promoting effects. Based on this trend, advances in the development of new food products with functional properties have been continuously increasing. Therefore, the demand for sensory tests to estimate the opinions of potential consumers and obtain reliable perceptions of the product is increasing [16].

Thus, the aim of this study was to assess the effects of the addition of a freeze-dried aqueous extract of tamarind seed on the antioxidant capacity and concentrations of total phenolic compounds in tamarind pulp. Nectars obtained from pulps to which aqueous tamarind seed extracts added were evaluated using sensory tests.

Material and Methods

Detailed descriptions of the "Material and Methods" are provided in a file in the Supplementary Material (please refer to the "Supplementary Materials and Methods").

Results and Discussion

Optimization of the Conditions Used to Prepare the Aqueous Extract

The concentrations of total phenolic compounds (TPCs) in the central composite design (CCD) ranged between 61.57 mg_{AG}/g_{dw} and 26.19 mg_{AG}/g_{dw}. Linear and quadratic models were created using these data and evaluated using analysis of variance (ANOVA). Neither quadratic nor interaction effects were observed; however, a linear regression analysis was sufficient to describe the data. Therefore, the generated linear model was able to predict the total phenolic contents in the aqueous extracts of tamarind seeds collected within the studied experimental region.

Supplementary Fig. 1 shows the generated response surface using the validated linear model. Time was the variable with the greatest effect on extraction. Longer times resulted in higher TPC contents in the aqueous extract. In contrast, the solid-to-liquid ratio (S/L) did not exert a significant effect, although a slight trend of improvement in TPC extraction was observed as this parameter is increased.

If the linear model is significant, it indicates that an optimum condition has not yet been achieved, revealing the necessity of extrapolating the ranges of the independent variables using a new CCD. However, as the extraction time was the only significant factor, this parameter was selected for a further univariate study. Hence, a new set of experiments with various extraction times, namely, 60, 80, 100, 120, 140 and 160 s, was conducted in triplicate. The results of these tests are shown in Supplementary Fig. 2.

An increase in the extraction time from 60 to 80 s positively affected TPC extraction from tamarind seeds, averaging a 10% improvement in the TPC content of the samples extracted for 80 s. In contrast, the TPC content started to decrease at 100 s, and samples extracted for 160 s contained up to 10% less TPC than samples extracted for 80 s. Based on these results, we set the extraction time to 80 s in the subsequent experiments.

Finally, the number of re-extractions, which is defined as the number of times that the same tamarind seed sample was extracted by replacing the extracting water, was studied. The results are shown in Supplementary Fig. 3. The TPC content decreased significantly as the number of re-extractions increased, and thus one re-extraction successfully extracted most of the TPC from the seeds. As excessive sample manipulation and time-consuming extraction protocols may favour antioxidant degradation, a 2-step extraction protocol (one re-extraction) was selected.

Therefore, the optimized conditions for preparing aqueous tamarind seed extracts were: an S/L ratio of 1:3 (seed:water), one re-extraction, and 80 s of extraction time. Tamarind seed powder, which was obtained after freeze-drying, had moisture content of 2 g/100 g on a humid basis and yielded approximately 25% dried solids compared to the fresh seeds. Finally, this powder (named FAE) was incorporated into fresh pulp at different concentrations to obtain the mixture that was used to prepare tamarind nectars.

Phenolic Compounds and Antioxidant Capacity of Methanol Extracts

The TPC contents and antioxidant capacity of methanol extracts of fresh pulp, fresh seeds, FAE and the mixture are described in Supplementary Table 1. Among these samples, fresh seeds and FAE contained the highest TPC concentrations of 365 and 475 mg_{GA}/g_{dw}, respectively, whereas the pulp had the lowest concentration, regardless the batch and the geographic origin, with a minimum value of 6.94 mg_{GA}/g_{dw} for P_{MG}.

The geographic region significantly affected the TPC content in the pulp, with P_{BA} showing the highest concentration (21.73 mg_{GA}/g_{dw}) among the samples. The batch effect was significant for tamarind pulp samples from São Paulo (P_{SP}) and Minas Gerais (P_{MG}), with batch 1 showing higher TPC concentrations for both geographic regions (11.49 and 7.63 mg_{AG}/g_{dw}, respectively). In contrast, pulp samples from Bahia did not show any significant differences between batches 1 and 2 (21.73 and 21.35 mg_{GA}/g_{dw}, respectively).

Similarly, seed samples from Bahia (S_{BA}) contained the highest TPC concentrations (359.39 and 365.16 mg_{GA}/g_{dw} for batches 1 and 2, respectively), whereas S_{SP} and S_{MG} did not differ significantly from one another. Razali et al. [17] reported lower TPC values in methanol tamarind pulp (3.35mg_{GA}/g_{dw}) and seed (272 mg_{GA}/g_{dw}) extracts produced in Brazil than the values reported in the present study. Tril et al. [18] observed lower concentrations in methanol extracts of seed and pulp (3.35 and 272 mg_{GA}/g_{dw}, respectively) from tamarind produced in Brazil than the values reported in the present study.

The higher reducing properties of the fresh pulp and seed from Bahia were reflected in the FAE produced, as it had the highest TPC content among all FAE samples (470.02 mg_{GA}/g_{dw}), followed by FAE_{SP} (429.34 mg_{GA}/g_{dw}) and FAE_{MG} (357.41 mg_{GA}/g_{dw}). Based on these results, the produced FAE may be used to increase the amount of antioxidant compounds in the pulp and to enrich other food products lacking these substances. This hypothesis is supported by the observation that mixed samples showed a significant increase in the TPC content compared to the pulp lacking the FAE. Regardless of the batch, more TPC was detected in the produced mixture as more FAE was added. For instance, when 0.3% FAE was added to the pulps from Minas Gerais, São Paulo and Bahia, the resulting mixture contained an average of 60, 50 and 28% more TPC than the fresh pulp without added FAE. In turn, following the addition of the second concentration (1.15%), an average increase of 260, 265 and 117% was observed, respectively, whereas the addition of the highest concentration resulted in increases of 465, 400 and 200%, respectively.

No effect of the harvest year (batches 1 and 2) was observed on the TPC content of mixed samples. Moreover, the mixture obtained using P_{BA} and FAE_{BA} displayed the highest TPC content at all concentration levels, except that the TPC contents of BA [2] and MG [3] were equal. The observed differences in the TPC concentrations between samples from different geographic regions may be attributed to edaphoclimatic factors [19].

Supplementary Table 1 also shows the results of the antioxidant assays. Among the studied tamarind fractions, pulps showed the lowest antioxidant capacity in all antioxidant assays.

Among the pulps, no significant difference was observed between batches 1 and 2 of samples with the same geographic origin, except for P_{MG} (71.4 and 105.6 mM_{Trolox}/g_{dw} for batches 1 and 2, respectively). P_{BA} had the highest antioxidant values for all assays, except for batch 2, which was statistically equivalent to P_{SP} in the ABTS assay (29.2 and 25.3 mM_{Trolox}/g_{dw}, respectively). In the FRAP assay, no significant difference was observed between batches 1 and 2 of P_{MG} and P_{SP}; however, the values of these pulps were approximately three times lower than batches of P_{BA}. In turn, in the ORAC assay, P_{MG} exhibited the lowest antioxidant activity (71.4 and 105.6 mM_{Trolox}/g_{dw} for batch 1 and batch 2, respectively), whereas in the DPPH assay, P_{SP} was the sample that showed the lowest radical scavenging capacity, with values ranging between 26 (batch 1) and 16.9 mM_{Trolox}/g_{dw} (batch 2).

Regarding the seeds, in general, S_{BA} showed the highest values in the ABTS, ORAC and DPPH methods for most batches. Interestingly, comparable antioxidant capacities were obtained using ABTS and ORAC methods, but the same trend was not observed in the pulps (Supplementary Table 1). The antioxidant substances present in the seeds potentially responded well both to the hydrogen atom donation mechanism (ORAC predominant mechanism) and the electron donation mechanism (ABTS), whereas antioxidants in pulps preferentially responded to ORAC-predominant mechanism [15].

FAE_{BA} showed the highest values in the FRAP, DPPH and ABTS assays; however, batch 1 of this sample did not differ from FAE_{SP} (2619.1 ± 358.8 mM_{Trolox}/g_{dw}) in the ORAC assay, whereas batch 2 (2241.2 ± 157 mM_{Trolox}/g_{dw}) produced significantly lower values than FAE_{SP} (2681.5 ± 254.0 mM_{Trolox}/g_{dw}).

In the mixed samples, the lowest values observed in the FRAP assay were recorded for batch 1 of SP [1] (28.9 mM_{Trolox}/g_{dw}), although a significant difference was not observed between this sample and BA [1] (36.5 mM_{Trolox}/g_{dw}). Similar activity was observed in batch 2, in which SP [1] (53.5 mM_{Trolox}/g_{dw}) did not differ from BA [1] (39.1 mM_{Trolox}/g_{dw}).

According to the results (Supplementary Table 1 and Supplementary Fig. 4), the mixed samples exhibited a higher antioxidant capacity than the pulp alone, suggesting that tamarind seed extracts may be an efficient alternative to increase the antioxidant properties of food formulations. Generally, the antioxidant capacity increased with the addition of increasing concentrations of FAE. For instance, for the radical scavenging capacity measured using the ABTS assay, the lowest increase in the antioxidant capacity was observed for SP [1] and BA [1], with values that were 194 and 137% higher than the values recorded for pulp without added FAE. Moreover, at the highest concentration (MG [3], SP [3], and BA [3]), an average increase of 1910, 1300 and 937%, respectively, was observed in the antioxidant capacity. For the DPPH method, the lowest increases were 88, 113 and 8% for SP [1], MG [1] and BA [1], respectively.

Interestingly, a lower increase in the antioxidant capacity of the samples was observed using the ORAC method (162, 201 and 125% for MG [3], SP [3], and BA [3], respectively), possibly because, as previously hypothesized, antioxidant substances in the seeds may present poorer hydrogen atom donation properties than antioxidants in the pulps, as reflected by a lower increase in the antioxidant capacity following FAE addition.

Because antioxidant assays measure different mechanisms of radical deactivation and antioxidant species in samples may function through distinct pathways to exert synergistic and antagonistic effects, a substantial variation in the antioxidant response is expected, even when the same food matrix is considered, as reported in previous studies of lemon (*Citrus limon*) [20], tamarind (*T. indica*) [18] and avocado (*Persea americana* Mill) [21]. This finding, combined with differences in fruit characteristics and distinct harvest times, may at least partially explain the variability in the antioxidant capacity observed in the present study, as well as the differences in the magnitude of increase in the antioxidant capacity.

Ultimately, tamarind seeds possess a high bioactive potential, suggesting that aqueous tamarind seed extracts have great potential to be used in food formulations, with the aim of increasing their functional properties.

Principal Component Analysis (PCA)

The results of the principal component analysis are shown in Supplementary Fig. 5. PC1 and PC2 were able to explain 97.9% of the variance in the data. In Supplementary Fig. 5A, three main groups are observed. Group 1 corresponds to pulp and mixed samples, group 2 contains seeds, and group 3 contains FAE samples. By analysing the graphs in Supplementary Fig. 5A and 5B, samples were distinguished according to their antioxidant capacities and concentrations of total phenolic compounds, with no separation according to the batch and geographic region. The high loading values in PC1 indicated that samples in groups 2 and 3 had a higher antioxidant capacity and concentrations of total phenolic compounds than samples in group 1. In other words, the seed contains more antioxidant substances than the pulp. High negative values in PC 2 for TPC and DPPH revealed that these variables contributed to discriminate the seeds, indicating that seed samples had the highest antioxidant properties among the other samples measured using these assays. In turn, FAE samples were differentiated from the other samples mainly by their ABTS and FRAP values, indicating that these samples showed a higher antioxidant capacity when assessed using these methods.

Because no separation of pulp samples was achieved, we decided to evaluate them separately by applying a new principal component analysis after excluding FAE and seed samples. New graphs were generated (Supplementary Figure5C and 5D), in which the new components were able to explain 96.18% of the variance in the data. As a result, the new PCA analysis revealed a clear discrimination trend between mixed samples to which different FAE concentrations were added, confirming that the incorporation of a higher concentration of the aqueous tamarind seed extract into the pulp resulted in a greater increase in the antioxidant property. Thus, the PCA results support our findings that the enrichment of tamarind pulp with FAE is an interesting alternative to improve its bioactive properties.

Sensory Analysis

Difference from the Control Test

The scores for appearance, aroma and flavour in the tamarind nectar produced in the states of Minas Gerais, São Paulo and Bahia enriched with different concentrations of FAE are presented in Supplementary Table 2. Among the samples from Minas Gerais, MG [1] was the only sample that did not differ from the control MG [0] in appearance and taste, receiving an approximate score of 1.81 ± 1.42. In contrast, the MG [2] and

MG [3] samples, which contained higher concentrations of the extract, differed significantly from MG [0] in both attributes, with mean scores of 2.55 ± 1.69 and 3.16 ± 1.90 for appearance and 2.90 ± 1.56 and 4.00 ± 1.90 for flavour, respectively. Regarding the aroma, the only treatment that differed from the control was MG [3], which contained the highest concentration of FAE, resulting in a mean score of 2.45 ± 2.13.

The appearance of samples of nectar containing pulp and FAE from the State of São Paulo was significantly different from the control. The sample SP [1] containing the lowest concentration of FAE received the minimum score (2.10 ± 1.45), and the highest score was recorded for SP [3] (3.52 ± 1.65), which contained a higher concentration of FAE.

Only SP [1] was not significantly different from the control in terms of the aroma, with a score equal to 1.19 ± 1.30. Regarding the flavour, the SP [1] sample also did not differ from the control, indicating that after the minimum addition of 0.6 g of FAE to 1 l of nectar, the flavour tended to differ from the control sample. The highest value of the minimum significant difference identified was 0.98 for the flavour. For appearance and aroma, the values were lower, 0.79 and 0.71, respectively.

The treatments with fruits from Bahia presented significant differences in appearance compared to the control, as scores of 3.52 ± 2.22, 3.16 ± 1.86 and 4.00 ± 2.07 were recorded for BA [1], BA [2] and BA [3], respectively, while the standard sample had a score of 1.06 ± 1.73, which was significantly lower than the other samples. The addition of FAE to the nectar at a concentration of 2.3 g_{FAE}/L (BA [2] and BA [3]) resulted in a significant difference in taste and aroma, and the BA [1] sample (0.6 g_{FAE}/L) was not distinguished from the control. The highest score was attributed to the BA [3] treatment at 4.42 ± 2.01, indicating a greater difference from the standard that was classified as moderately different to very different from the control.

In general, when the concentration of FAE in the seed is increased in the nectar, the scores tended to be higher, indicating an increase in the degree of difference of the attributes compared to the control sample. The maximum amount of FAE that can be added to prevent differentiation from the control is 2.3 g_{FAE}/L for fruits from any state of origin.

Acceptance Test

Although judgments have oscillated between "dislike moderately" (4) and "like moderately" (6), regardless of the geographical origin or concentration of FAE added, the high variance in scores between judges (40 to 50%) indicated that consumers preference for tamarind nectars varied substantially. This finding was confirmed by scores for the control sample (without added FAE), which showed similar variance in the data.

Regarding the appearance, scores were centred on "neither liked nor disliked" (5). SP [3] nectar received the lowest score (4.51 ± 2.24) among all samples. For the attribute aroma, MG [3] had the lowest score, whereas BA [0] had the highest score (5.44 ± 2.21). However, the substantial variance in scores between judges resulted in a non-significant difference in this attribute between samples.

Overall, samples did not differ significantly one from another in flavour. However, given the absolute lower scores for MG [3], SP [3] and BA [3], we concluded that a tendency of poorer flavour acceptability occurred as the FAE concentration in nectars increased. A similar trend was observed for texture and the global impression, as the acceptance by the judges decreased as the FAE concentration increased. Based on these results, food products with better functional properties are not necessarily the most adequate for commercialization without a risk of suffering rejection from consumers. Therefore, they reinforce the importance of combining technological information and data from sensory analyses during product development.

Purchase intent was another test performed to evaluate the acceptance of the nectars. Generally, scores oscillated between 2, 3 and 4. Nectars from Minas Gerais received the worst scores, as "I certainly would not buy" and "possibly would not buy" were the most frequently recorded judgements. However, SP [3] and BA [3] were evaluated better, as "possibly would buy" and "certainly would buy" were the most frequent judgements recorded for these samples.

Conclusions

In the present study, we produced a freeze-dried aqueous extracts of tamarind seed and incorporated them into tamarind pulp, with the aim of increasing the concentrations of phenolic compounds and antioxidant capacity. Tamarind pulp possesses lower levels of phenolic compounds and antioxidant capacity than the seed. Moreover, for both the pulp and seed, these properties varied significantly according to the geographical region and batch. When added to the pulp (mixture), the freeze-dried aqueous extract of tamarind seed significantly increased the content of phenolic compounds and antioxidant capacity. Namely, the incorporation of higher concentrations of the seed extract increased these properties in the mixed samples. Sensory tests showed that the concentration of FAE added was the key factor contributing to the judges' perceptions of any sensorial difference in nectars. Overall, low to intermediary concentrations of FAE are recommended to be incorporated into the pulp to generate a highly acceptable nectar. Thus, tamarind seeds are a valuable source of bioactive compounds and have the potential to enrich fruit products to enhance their health appeal and increase their market value.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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