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Effect of roasting and soaking on Proximate Composition, Anti-nutritional, Antioxidant and Antimicrobial properties of Tamarind Seed Powder

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Abstract: *The research isprimarily focused on analyzing the effect of different processing methods i.e. soaking (cold and hot) and roasting of theTamarind indicaL. seed. Differently processed samples were subjected toanalysis such asproximate composition, anti-nutritional factor, antioxidant activity, phenolic content and antimicrobial activity. Anti-nutritional factors i.e., tannins, alkaloid, saponin and phytate, were significantly affected ($p \leq 0.05$) by different processing. Tannin, alkaloid, saponin and phytate content of samples were found in the range of 4.05-20.04 %, 3.22-3.49 %, 0.5-1.04 and 2.10 - 2.92 % respectively.Free radical scavenging activity ranged from 16.07 ± 0.98% to 75.14 ± 1.34%, with all sample being significantly influenced ($p \leq 0.05$) by theapplication of different methods. The phenolic content intamarind seed for samples were in the range of 9.55 - 52.33mg/100ml respectively. Total antioxidant capacity was found to be positively correlated with total phenolic content having $R^2 = 0.9467$. The antimicrobial test was carried out for Staphylococcus aureus, Bacillus subtilis, E. coli, and Salmonella typhi. Among the samples, roasted sample was found to be most effective against tested micro- organisms.*

Keywords: *Tamarind, Proximate composition, Antioxidant activity, Anti-nutritional factor, Antimicrobialactivity*

Introduction

Tamarind (*Tamarindusindica* L.), taxonomically belongs to a class of dicotyledon and fabaceae family (Chant, 1993). It is grown in over 50 countries around the globe and the primary producers are India, Bangladesh, Sri Lanka, Thailand, and Indonesia in Asia, as well as Africa and the Americas. In Nepal, it is commonly known referred to*'imli' or 'titri'*. The tamarind tree is cultivated in limited scale and also grows in wild. It's a big, evergreen or semi-evergreen tree with a long lifespan. A mature tree can grow up to 30 meters tall (Jambulingam and Fernandes, 1986; Stross, 1995) and it has the ability to thrive in adverse conditions such as drought and in infertile soil because of its capability to fix nitrogen (Felker, 1981; Felker and Clark, 1980).

Primarily, fresh fruits of Tamarind (*Tamarindusindica* L) are eaten and if processed they are used as a seasoning or spice. The pod pulp (40 percent) is rich in vitamin C and contains dihydroxybutanedioc acid, malic and citric acids along with some sugars. It has a sweet-sour taste and is used as ingredients in beverages, sweet meats, curries and chutneys and is a necessary component of Worcestershire sauce. Tartaric acid, also known as dihydroxybutanedioc acid, is the primary acidulant used in Indian food products, and huge amount of tartaric acid is present in pulp of tamarind. Almost every component has at least one use, whether in textiles, carpentry, nutrition, or medicine.Since almost every aspect of the tamarind tree is useful, it is known as a multipurpose tree.

Tamarind fruit-is pendulous- consists of pulp (55%), seed (34%) and shell (11%) as well as the fiber in a pod (Rao and Srivastava, 1974). Shell is porous in nature and easily breaks when squeezed. The pulp seems dense and brownish-black in appearance (Coronel, 1991; Purseglove, 1987).

Tamarind seed extract have antioxidant properties and antimicrobial properties (Tsuda et al., 1994). Varieties of phytochemicals are present in seed of tamarind (Andabati and Muyonga, 2014)."It also consists of phenolic antioxidants such as 2-hydroxy-3', 4' dihydroxy acetophenone, methyl-3, 4-dihydroxybenzoate, epicatechin, and 3,4-duhydroxyphenyl acetate" (Sudjaroen *et al*., 2005; El-Siddig *et al.,* 2006). As a result, tamarind seed has the opportunity to enhance to low-cost nutrition with nutritional supplement benefit.

Tamarind also known as *Imli* is emerging in Nepal but has not received much attention. Plant proteins, minerals, and vitamins B and C are all contained in tamarind seed powder. In context of Nepal, people rarely know the importance of seeds and its proper processing for the consumption. There has been little research on various processing treatments that appear to reduce anti-nutritional factors in tamarind seed. Despite the utilization and availability of *Tamarind indica* in Nepal, little is known on the amount and activity of antioxidants, anti-nutritional and antimicrobial properties from this plant. Furthermore, there is limited information on comparative analysis of antioxidant compounds, anti-nutritional compounds and antimicrobial properties of tamarind seed powder. Therefore, this study is aimed to fill the existing knowledge gap. Findings from the study would be useful in providing baseline information about the result ofdifferent processing treatment on antioxidant, anti-nutritional and antimicrobial properties of tamarind seed powder.

The aim of this analysis was to see how different processing methods affected the results on proximate composition, anti-nutrient contents,and antimicrobial properties of tamarind seed powder.Further, this research will also help to scrutinize the effect onantioxidant activity and phenolic content of tamarind seed powder withvarious processing methods.

Materials and methods

Collection Raw materials

The key raw material used to determine the effects of various processing treatments was the tamarind (*Tamarindusindica* l.), which was procured from local market of Kathmandu. The utensils, glassware and other requiredequipment's were used from College of Applied Food and Dairy Technology (CAFODAT).

Method of sample preparation

Roasted sample

Tamarind seeds (200 g) were dehulled after being roasted in a pan at 110°C for 15 minutes. The dehulled roasted seeds were ground into flour with a grinder and dried in the light. To obtain fine flour, the sample was sieved at 150 mesh size and packaged in a pouch for further study.

Soaked sample

Cold soaking

To remove the seed coat, 200 g of tamarind seed was immersed in cold water at ambient temperature for a day. The seeds were thoroughly dried in sun and ground. To obtain fine flour, the sample was sieved at 150 mesh size and packaged in a pouch for further examination.

Hot soaking

Tamarind seed (200 g) was soaked in water and maintained at temperature of 80°C for 15min that facilitated in removal of seed coat. The seeds were dried in the sun and ground.The sample was sieved at 150µm mesh size to obtain fine flour and packaged in a pouch for further analysis.

ProximateAnalysis of *Tamarindusindica*L. seed powder

Tamarindusindica's moisture content was determined using the Dean and Stark instrument and the solvent extract method.Protein content, fat content, ash and crude fiber was determined as described in AOAC, 2005.Carbohydrate content was determined by differential method (Pearson, 1976), using formula: Carbohydrate = 100 - (Ash % + moisture % + crude protein % +crude fat % + crudefiber %).

Determination of Anti-nutritional factors

Tannin

In a conical flask, 20g of the processed sample was mixed with 100ml of petroleum ether for 24 hours. The sample was filtered, and the filtrate was left to sit until all of the petroleum ether had evaporated. It was re-extracted by soaking it for 4 hours in 100ml of 10% acetic acid in ethanol. The filtrate was obtained after the sample had been purified once more. With the addition of 25mL of NH_4OH to the filtrate, alkaloid precipitation was achieved. To remove a few of the remaining NH_4OH, the alkaloid was heated on a hot plate. It was taken 5mL of this and mixed with 20ml of ethanol. The solution was then titrated with 0.1M NaOH and phenolphthalein until it reached the pink end-point (Pearson's 1976). Tannin content was measured as a percentage using the following formula: (C1V1=C2V2) Molarity.

Where,

C1 – tannin acid concentration

V1– volume of the tannin = 5ml

V2– volume of the base = titer

C2– concentration of the base = 0.1 N

Therefore, C1 = (C2*V2) / V1

percent of Tannin acid content = (C1 – 100) / weight of analyzed sample

Phytate "0.2 g of each processed sample was weighed into a 250 mL conical flask. Each sample was immersed in 100 mL of 2 percent concentrated HCL for 3 hours. Following that, the sample was filtered. Each sample

was put in a 250 mL beaker with 50 mL filtrate and 100 mL distilled water. As an indicator, 10 mL of 0.3 percent ammonium thiocyanate solution was added and titrated with a regular Iron (iii) Chloride solution containing 0.00195 g Iron per mL. According to Akajiaku *et al.,* (2014), the percentage phytic acid was determined using the following formula:

Phytate acid (%) = (titre value × 0.00195×1.19×100) / 2

Alkaloid

In 250 mL beaker,"5 g of test sample was weighed and 200 mL of 10% acetic acid in ethanol was added, covered, and left to stand for 4 hours. The extract was evaporated to one fourth of its original volume in a water bath after the suspension was filtered. Drops of concentrated ammonia aqueous were applied to the extract until it was fully precipitated. After washing with dilute ammonium hydroxide and allowing the solution to settle, the precipitate was collected and filtered. The residue of alkaloids was allowed to dry and weighed (Harborne, 1973).

Saponin

In 100 ml of 20 percent ethanol, 5 g of each sample was dispersed. The suspension was heated for 4 hours at 55 degrees Celsius in a hot water bath with constant stirring. With 100 mL of 20 percent ethanol, the filtrate and residue were re-extracted. Over a water bath at around 90 degrees Celsius,"the combined extracts were reduced to 40 ml. The concentrate was moved to a beakerand vigorously shaken with the addition of 20 mL of diethyl ether. Two layers -Aqueous layer and ether layer-was obtained and the former was recovered while the latter was discarded. 30 mL of n-butanol was added to aqueous layer and shaken for the purification process. N-butanol combines with the extract so, to wash this solution 10 mL of 5% aqueous sodium chloride was used. Washing was done twice and the remaining solution was heated in a water bath. After evaporation, drying of the sample was carried out in the oven to a constant weight and thesaponin content was calculated as percentage (Obadomi and Ochuko, 2001)."

Antioxidant activity

To determine antioxidant activity, DPPH assay was used. 1.0 ml of DPPH solution (80 g/ mL ethanol) was mixed with 0.2 ml of sample suspension. The reaction mixture was then left to stand at ambient temperature for 30 minutes, shaking twice or three times in between. In aliquet of a sample, distilled water was used as a blank. The tubes were then centrifuged at 3000 rpm for 10 minutes to separate the clear supernatant. Spectrophoto meter was used to measurethe absorbance of the sample mixture at 517 nm after diluting the clear supernatant with ethanol. The decolorizing degree of the absorbance difference between the blank and sample suspensions was used to determine the degree of scavenging.

Scavenging activity= (1- A2/A1) × 100

Where, A1= Absorbance of blank sample, A2= Absorbance of sample

Total phenolic content

Folin-Ciocalteau reagent and Gallic acid standard were used to assess the total phenolic content suggested by (Druckerei, 2002) and (Jayaprakash *et al.*, 2003).

Antimicrobial activity

Using the agar well diffusion method, antimicrobial activity of variously processed seeds was tested to *E. coli, Staphylococcus aureus, Salmonella typhi,* and *Bacillus subtilis*.

Data analysis and interpretation

The obtained data were subjected to analysis of variance, least significant difference and Duncan test at significance level p=0.05 using SPSS ver.16 and the graphs were plotted using Microsoft excel (2016).

Results and discussions

Proximate Composition of Tamarind seed Powder

Table 1: Tamarind seed Powder's Composition

Parameter	Values obtained			
	Raw	Roasted	Cold Soaking	Hot Soaking
Moisture%	8.70^{c} ± 0.23	5.64^{d} ± 0.07	11.73^{a}± 0.09	10.66^{b} ±0.27
Protein%	23.78^{b} ±0.33	20.83^{a} ± 0.64	23.70^{b} ± 0.47	19.05^{c}± 0.83
Fat%	9.69^{a} ± 0.11	6.62^{c} ± 0.40	7.77^{b} ± 0.37	4.66^{d} ± 0.25
Ash%	2.55^{b} ± 0.10	2.67^{a}± 0.09	1.76^{b} ± 0.18	2.10^{a} ± 0.03
Crude fiber%	6.12^{c} ± 0.02	6.35^{a} ± 0.04	6.16^{c} ± 0.02	6.25^{b} ± 0.02
Carbohydrate%	49.16^{b} ±0.21	57.89^{a} ± 0.24	48.88^{b} ± 0.13	57.28^{a}± 0.40

*The values in the table are arithmetic mean ± SD of three replicates. All the parameters are in dry basis except moisture and carbohydrate. In a row, the values of the same superscript letters do not differ significantly (p>0.05).

Moisture content

Moisture content of the cold soaked tamarind seed was found to be highest whereas that of raw sample was lowest. The percent moisture content of the tamarind seed was found in range of 5.64- 11.73%. Akajiaku *et al.*, (2014) reported the moisture content for seed nut range from 8.0% to 10.5%. Moisture content of the samples wereaffected significantly ($p \leq 0.05$).

Protein content

The protein content of raw, roasted, cold soaked and hot soaked tamarind seed was found in the range of 19.05-23.78 %. The loss of soluble protein during soaking may have resulted in a decrease in protein content in processed samples. However, between cold soaked and raw sample, statistical analysis result revealedno any significant effect ($p > 0.05$) in protein content. Roasted sampleand hot soaked sample, however, were significantly affected ($p \leq 0.05$). Protein reduction during roasting can indicate the Maillard reaction, as protein is non-enzymatic browning substrate (Tenyang *et al.,* 2017). Arinola and Adesina (2014) ascribed similar result who reported that during processing treatment i.e., boiling and roasting, the amount of protein of walnut seed reduced.

Fat content

The highest fat content was found in the raw sample, while the lowest was found in the hot soaked sample. The fat content of the tamarind seed powder for raw, roasted, cold soaking and hot soaking were found to be 9.69 ± 0.11, 6.62 ± 0.40, 7.77 ± 0.37 and 4.66 ± 0.25, respectively. The statistical studyshowed that processing had a significant influence ($p \leq 0.05$) on the fat content of samples. Similar results i.e., decrease in fat content in asparagus bean is reported by (Nzewi and Egbuonu, 2011).

Ash

The ash content of the tamarind seed for samples were found in the range of 1.76-2.67%.The value of ash content for raw sample was very close to (Mishra *et al.,*2018) who stated the value as 2.60 ± 0.08 %. Cold soaking had no significant effect ($p>0.05$) but roasting and hot soaking significantly influenced ($p \leq 0.05$) ash content. The part of food or other organic material that remains after being burned at extremely high temperatures is referred to as ash or mineral content. The value of soaked sample was less than that of untreated (raw) sample; the decrement of the ash content is due to the leaching of major and minor elements during soaking (Siddhuraju *et al.,* 1995), whereas that for roasting was found to be little higher than untreated sample. Raw seeds have a lower ash content than roasted seeds, which may be attributed to anti-nutrient effects on the mineral content of the food sample. (Nwafor *et al.,* 2016).

Crude fiber

The crude fiber of the tamarind seed for raw, roasted, cold soaking and hot soaking were found to be 6.12 - 6.35 %. According to statistical analysis, no significant effect ($p > 0.05$) between cold soaked and raw sample was observed. However, the crude fiber content of roasted and hot soaked sample was significantly influenced ($p \leq 0.05$). Crude fiber content of the raw sample was similar to that of (Mishra *et al.,* 2018), who recorded a crude fiber content of 7.00 ± 0.08 %.The crude fiber composition of the hot soaked sample was higher than the cold soaked sample, insinuating that more crude fibers, during boiling were likely leached into the water (Aremu *et al*., 2006).

Carbohydrate

The carbohydrate of tamarind seed for raw, roasted, cold soaking and hot soaking were found to be 49.16 ± 0.21, 57.89 ± 0.24, 48.88 ± 0.13 and 57.28± 0.40 respectively. Between cold soaked and raw sample, no significant effect ($p > 0.05$) of processing was observed. Roasting and hot soakingtreatments, however, significantly affected ($p \leq 0.05$) the carbohydrate content. The carbohydrate value corresponded to that reported by Akajiaku *et al.* (2014), which was 56.24% – 58.08%.

Antinutritional factors:

Table 2: Anti-nutritional component of Tamarind seed

Anti-nutritional factors (%)	Values obtained			
	Raw	Roasted	Cold Soaking	Hot Soaking
Tannin	20.04^{a} ± 0.16	8.33^{b} ± 0.10	4.82^{c} ± 0.30	4.05^{d} ± 0.05
Alkaloid	3.49^{a} ± 0.06	3.37^{b} ± 0.04	3.40^{b} ± 0.30	3.22^{c} ± 0.05
Saponin	1.04^{a} ± 0.05	1.02^{a} ± 0.05	0.79^{b} ± 0.03	0.5^{c} ± 0.04
Phytate	2.92^{a} ± 0.05	2.42^{b} ± 0.11	2.15^{c} ± 0.06	2.1^{c} ± 0.03

*The values in the table are arithmetic mean ±SD of three replicates. The values of the same superscript letters in a row do not vary significantly ($p>0.05$).

Tannin content

The tannin content of samples was found in the range of 4.05-20.04%. The value of tannin content for treated sample was within the range 4.84%-8.34% (Akajiaku *et*

al., 2014). El Anany (2015) suggested that with proper application of heat treatmenti.e roasting in guava seed, leads to reduction in the tannin content. El Maki *et. al., 2007,* also reported reduction in tannin level in white bean due to soaking and cooking. Since tannins are water soluble (Kumar *et al.,* 1979) in nature, they may have leached out during soaking thus reducing the tannin content of soaked samples. Various treatment processes namely soaking and roasting, statistically, significantly affected the tannin content of tamarind seeds ($p \leq 0.05$).

Alkaloid content

The alkaloid content intamarind seed of different samples were found in between 3.22- 3.49 %. The alkaloid content of the samples was significantly influenced ($p \leq 0.05$) by the processing methods. However, there was no substantial difference between the roasted and cold-soaked samples ($p>0.05$). Yadesa and Biadge, (2017) suggested decreasing trend in the alkaloid content in lupin bean by the different processing treatments. Similarly (Justina Y. Talabi *et al.*, 2016), also reported the reduction of alkaloid of Avocado (*Persea americana Mill*) seeds with different processing.

Saponin

The saponin content of the tamarind seed for raw, roasted, cold soaking and hot soaking were found to be 1.04 ± 0.05, 1.02 ± 0.05, 0.79 ± 0.03 and 0.5± 0.04 % respectively. There was no significant effect ($p > 0.05$) on saponin content for roasted sample. Soaking, however, significantly influenced ($p \leq 0.05$) the saponin content. This finding agrees with the findings of (Ihemeje *et al.,* 2018), who found that heat and soaking reduced saponin content in African yam bean and red kidney bean.

Phytate

The phytate content in tamarind seed for raw, roasted, cold soaking and hot soaking were in the range of2.10 - 2.92%. Ihemeje *et al.,* (2018), who reported the reduction of phytate content in African yam bean and red kidney bean with application of heat and soaking, obtained similar result. The phytate content was significantly affected ($p \leq 0.05$) by roasting and soaking, according to statistical analysis. However, no substantial difference ($p > 0.05$) was observed between cold and hot soaked samples. During processing, insoluble compounds such as phylate proteins and phylate mineral compounds are formed which is responsible for reduction of phytate content (Vijayakumari *et al*., 1997).

Total antioxidant activity

The free radical scavenging ranged from 16.07 ± 0.98% to 75.14 ± 1.34% and all samples differed significantly ($p \leq 0.05$) from one another. The results for samples were in the order of Roasted > hot soaked > cold soaked > Raw. The value of DPPH inhibition percentage for raw was lower than the value suggested by Luzia and Jorge, (2011). This lower value of raw sample might be due to the influence of geographical and climatic factor. Ghasemi *et al.,* (2011) reported in his research that significant difference can arise in content of bioactive compounds and their bioactivities due to environmental factors.

The treatment methods resulted in increased antioxidant activity according to the DPPH method. These findings correspond with Rocha-Guzmán *et al.,* who stated that free radicals are expelled at faster rate in cooked beans (treated) than untreated (raw) beans.The increment in antioxidant capacity following heat application with or without soaking, according to Huber *et al.*, (2016), may be attributed to high amount of phenolic compounds in the cooking broth, which promoted their extraction. Terpinc, Petra and Polak *et al.,* (2011) also found similar findings, indicating that heat treatment of camelina seed significantly improved antioxidant activity.

Table 3 : DPPH inhibition % and total phenolic content for raw, roasted, cold soaked and hot soaked Tamarind indica L. seeds.

Parameters	Raw	Roasted	Cold soaking	Hot soaking
Inhibition %	16.07^{d} ±0.98	75.14^{a} ±1.34	24.60^{c} ±2.12	64.27^{b} ±2.77
TPC (mg/100ml)	9.55^{d} ±0.70	52.33^{a} ±1.20	17.67^{c} ±1.14	35.73^{b} ±1.55

*The values in the table are arithmetic mean ± SD of three replicates. Values in rows with different superscripts are significantly different ($p \leq 0.05$).

Phenolic Content

The phenolic content of raw, roasted, cold soaked and hot soaked samples was found to be 9.55 ± 0.70, 52.33 ± 1.20, 17.67 ± 1.14 and 35.73± 1.55, respectively. Statistical analysis revealed that the phenolic content of the seedsi.e., cold soaked, roasted and hot soaked samples were significantlyinfluenced ($p \leq 0.05$) by heat treatment. There was high accretion of total phenolic content in hot soaked and roasted sample compared to cold soaked sample. This may be due to the heat treatment releasing available polyphenol compounds.

Active compounds that were previously tightly bound to the seed tissue may have converted to free formsdue to heating effect from hot soaking and roasting, resulting in improved antioxidant effects. Lim and Kim, (2018) also reported the increment in phenolic content of *Ginko biloba* seeds by heat treatment.

The samples having high phenol content had higher antioxidant capacity, which is shown in the figure 1. These results indicated that antioxidant activity is directly influenced by the phenolic content, which is further increased by heat treatment. Positive correlation with R^2 = 0.9467, was observed between antioxidant activity and total phenolic content.

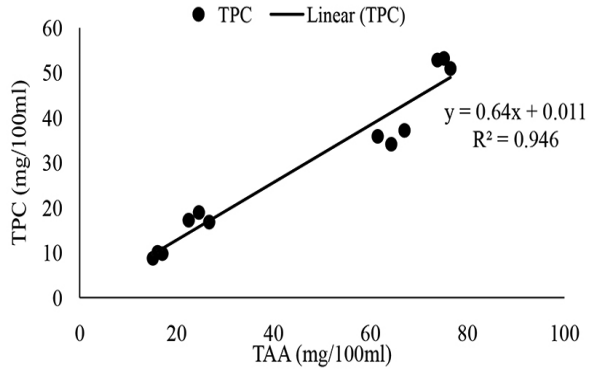

Figure 1: Correlation between antioxidant activity and total phenolic content (R2= 0.9467), TAA= Total antioxidant activity, TPC= total phenolic content

Antimicrobial activity

Four bacterial species were used and aqueous-ethanolic extract of tamarind seed was tested using the agar well diffusion method to determine the antimicrobial activity of tamarind seed. Table 4 shows the antimicrobial activity of tamarind seed extract against*E. coli, Staphylococcus aureus, Salmonella typhi, and Bacillus subtilis.*

The activity of the roasted sample against tested microorganisms were found to be higher compared to other samples. This might be because of the different chemical that might have released during roasting. The extract was effective against testedmicroorganism's that were procured from the laboratory of B and B hospital however; the antimicrobial activity was higher in gram-positive bacteria. Gupta *et al.,* (2014), in accordance with the earlier observations reported similar observations from the herbal extract of tamarind fruit. In comparison to gram negative bacteria, the seed extract was most effective against gram positive bacteria.

Table 4: Antimicrobial activity of differently processed seed

Sample / Micro organisms	Zone of inhibition (mm)			
	Staphylococcus aureus	*Bacillus subtilis*	*E. coli*	*Salmonella typhi*
Raw	13.13^{c}± 0.15	12.17^{d} ± 0.21	10.23^{d} ± 0.35	11.07^{d} ± 0.21
Cold Soaked	15.50^{b} ± 0.15	13.57^{c} ±0.15	12.93^{c} ±0.25	12.10^{c} ± 0.20
Hot Soaked	15.60^{b} ± 0.44	16.43^{b} ± 0.21	14.57^{b} ± 0.21	13.57^{b} ± 0.32
Roasted	18.07^{a} ± 0.25	17.23^{a} ± 0.35	16.13^{a} ± 0.15	16.60^{a} ± 0.36

*The values in the table are arithmetic mean ± SD of three replicates. Thevalueshaving same superscript letters in column do not vary significantly (p>0.05).

Conclusions

The processing methods (soaking and roasting) were found to improve the fiber content of the tamarind seed. Roasting significantly increased the ash content whereas there was decrease in protein content and fat content.The anti-nutritional content (tannin, alkaloid, saponin, and phytate) of the tamarind seed was significantly influenced by soaking and roasting.The processing treatment are efficacious and it can result in an improvement in nutritional quality due to the reduction of anti-nutritional components which reduces the bioavailability of minerals and other nutrients.The processing methods have a significant effect on the overall antioxidant activity and phenolic content of the seed. The antimicrobial activity shown by tamarind seed inhibited the growth of two gram-positive and two gram-negative bacteria andamong soaking and roasting method, roasting was most effective against these bacteria.

Recommendation

Based on the study conducted, further investigation can be performed to test the efficacy of the seed powder as a preservative in different food products, and also functional properties of tamarind seed powder can be studied which can further open new lines of inquiry in beverage formulation.

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