

Spectrophotometric Estimation of Tannins in Raw and Processed Form (Paan Masala) Of Areca Nut

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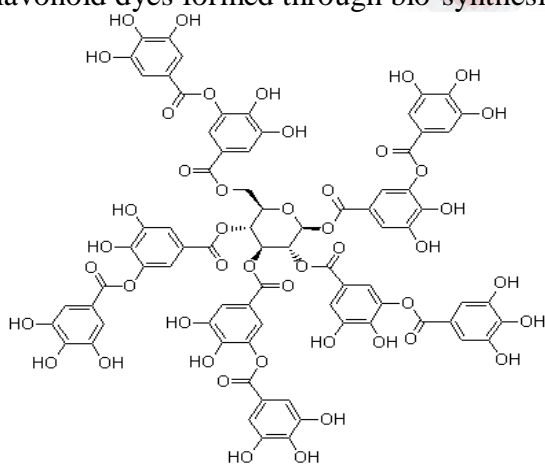
ABSTRACT

Tannins appear as light yellow or white amorphous powders or shiny, nearly colorless, loose masses, with a characteristic strange smell and astringent taste. In the present paper, tannin content in both raw and processed form (Paan masala) of areca nut was estimated using Folin-Denis reagent spectro photometrically and their comparative analysis was also done. Tannic acid was taken as a standard and values were expressed as tannic acid per 100 ml of the sample. The tannin content was found higher in processed forms than raw areca nut. It might be due to the presence of tannin in other components such as lime, areca nut, clove, cardamom, mint etc. in the processed form.

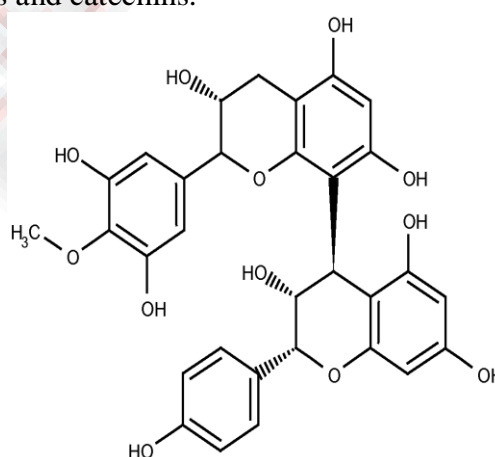
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1.1 INTRODUCTION

Tannins are astringent, bitter plant polyphenols that either bind and precipitate or shrink proteins and various other organic compounds including amino acids and alkaloids [1]. Tannins have molecular weights ranging from 500 to over 3,000 (gallic acid esters) and up to 20,000 (proanthocyanidins) [2]. Tannins can be classified into two broad groups - **hydrolysable tannins** and **condensed tannins**. Hydrolysable tannins are polymers of simple phenolic acids like gallic acid or ellagic acid esterified to a core molecule, commonly glucose or polyphenol such as catechinb [3-5]. Condensed tannins (non-hydrolysable tannins) do not split easily and are basically flavonoid dyes formed through bio-synthesis of flavins and catechins.



(a) Hydrolyzable tannins



(b) Condensed tannin

Figure1.1: Structure of tannins

Medically, tannins are used as antidotes to poisoning by alkaloids depending on their capacity to form insoluble tannates. The other remedial values of tannins include application on burns to heal the injury and on cuts to stop bleeding. Tannins can also be effective in curbing hemorrhages as well as restrict bare swellings. Herbs possessing tannins are widely used as mouthwashes, eyewashes, snuff and even as vaginal douches and also treat rectal disorders [6, 7]. Tannins are potential biological antioxidants used for defense of oxidative damage, which has been implicated in a wide range of diseases including cancer, cardiovascular disease, and arthritis and ageing [8]. Tannins also exhibit anti nutritional properties.

The present paper illustrates the total tannin content present in the different kinds of paan masalas purchased from different lots and different regions of Jaipur. The tannin content present these samples are compared with the tannin content present in raw areca nut.

1.2 Experimental

1.2.1 Sample collection

In order to estimate the total tannin content in raw and processed areca nut, firstly sample were selected on the basis of the commonly consumes paan masala by the Indians. These samples were purchased from four local areas (Mansarovar, Gopalpura, Badi Chopar and NandPuri) of Jaipur city. Mansarovar area was selected as standard area from where we purchased raw areca nut and compared it with all the processed form of areca nut obtained from different areas. For estimation of tannin in areca nut and its processed form (Paan masala) we use tannic acid as a standard. Tannin was determined using Folin-Denis reagent spectrophotometrically [9, 10]. Spectrophotometric estimation of tannin is based on the measurement of blue colour formed by the reduction of phosphotungusto molybdic acid by tannin like compounds in alkaline solution.

1.2.2 Reagents required:

- (a) **Folin-Denis reagent:** To 750 ml of water, 100 g of sodium tungstate ($\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$), 20 g of phosphomolybdic acid and 50 ml of 85% phosphoric acid (H_3PO_4) is dissolved. The mixture is refluxed for 2 hr, cool to 25 C and dilute to 1000 ml with water. Alternatively use readymade solution.
- (b) **Saturated sodium carbonate solution:** To 100 ml of water, 35 g of anhydrous sodium carbonate is added, dissolved at 70-80 ° C and cooled overnight. Clear liquid is decanted before the use.
- (c) **Tannic acid standard solution:** 100 mg of tannic acid is dissolved in 1 litre of water. Fresh solution is prepared for each determination (1 ml = 0.1 mg of tannic acid).

1.2.3 Procedure:

1 g of sample was boiled in 400 ml of water for 30 min. Transferred to 500 ml volumetric flask and diluted up to the mark. Shaked well and filtered.

1.3 Preparation of standard curve

1.3.1 Tannic acid standard solution:

100 mg of tannic acid is dissolved in 1 liter of water. Fresh solution is prepared for each determination (1 ml = 0.1 mg of tannic acid). Pipette 0 to 10 ml aliquots of the standard tannic acid solution into 100-ml volumetric flasks containing 75 ml of water. Add 5 ml Folin-Denis reagent and 10 ml Na_2CO_3 solution into each of the volumetric flasks and make up to 100 ml with water. An aliquot of the filtrate containing not more than 0.1 mg of tannic acid is used. Proceed as in standard, and obtain mg tannic acid from the standard curve (**Figure 1.2**). Samples were mixed well and the color was measured after 30 min at 760 nm against experimental blank adjusted to absorbency.

Table 1.1

Tannic acid (in ml)	Folin - denis reagent (in ml)	Sodium Bicarbonate (in ml)	Water (in ml)
0	5	10	85
2	5	10	83
4	5	10	81
6	5	10	79
8	5	10	77
10	5	10	75

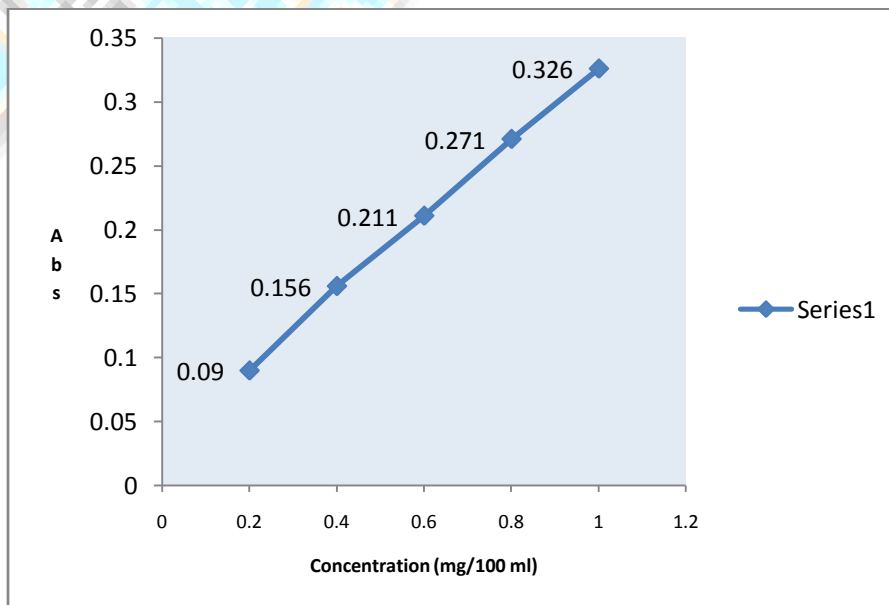


Figure 1.2 : standard curve between absorbance and concentration.

1.3.2 Calculation

$$\text{Tannin as Tannic acid \%} = \frac{\text{mg of tannic acid} \times \text{Dilution} \times 100}{\text{ml of sample taken} \times \text{wt. of sample taken} \times 1000 \text{ for color development}}$$

Table 1.2: Graphical comparison between absorbance and different areas of different samples

Samples	Area			
	Gopalpura	Badi Choupar	Nandpuri	Mansarovar
A	0.367	0.360	0.362	0.361
B	0.245	0.263	0.234	0.239

Table 1.3: Statistical comparison between mean and standard deviation

Samples	Mansarovar	Gopalpura	Badi Chaupar	Nandpuri
A	0.361±0.006	0.367±0.006	0.360±0.003	0.362±0.003
B	0.239±0.013	0.245±0.006	0.263±0.006	0.234±0.006

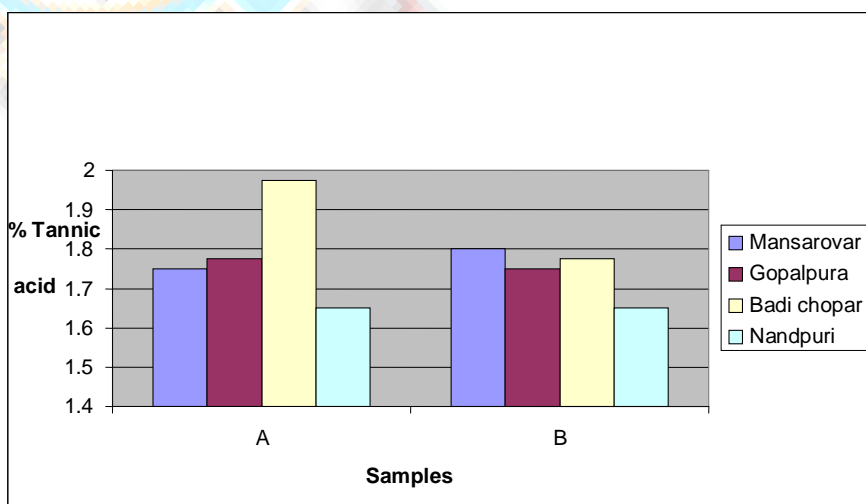


Figure 1.3: Graphical representation of % tannic acid in two different samples from various geographical locations.

RESULT AND DISCUSSION

The tannin content observed in raw areca nut was 1.7%. The tannin content of a nut may vary depending on the region where areca catechu is grown, its degree of maturity and its processing method. The tannin content is highest in unripe areca nuts and decreases substantially with increasing maturity. Both the samples were almost in the same range in all the areas. Processed sample values are approximately in the range of values of raw areca nut. The obtained percentage of tannin may vary a little because duration of research was from winter to summer so the temperature conditions were different throughout the experimentation. Slight variations may also be due to the presence of other components viz. lime, areca nut, clove, cardamom, mint, tobacco and essences in the processed form.

1.5 CONCLUSION

The present study was undertaken with the purpose of estimating the total tannin content of raw and processed forms of areca nut and to assess the changes in tannins, if any, due to processing. For the present study the samples were purchased from four places in Jaipur-Mansarovar, Gopalpura, Badi Chopar and Nandpuri. Estimation of total tannins was done for raw and processed forms of areca nut. The total tannin content of raw areca nut was found to fall in the range of 1.5-1.8% whereas the total polyphenol content of processed forms was found to fall in the range of 1.7-2.9%. The tannin content of raw areca nut was less as compared to processed forms of areca nut. The difference in the data obtained in this study and that reported in the literature may probably be due to several reasons such as differences in the variety, maturity, environmental condition in which they are grown, storage etc. in the raw form of areca nut. In the processed form extraction procedure, method of analysis and the standards used to express the results contributed to the variation. Although the size and variability of samples make the study difficult, to fully estimate the amount of tannins, it is necessary to investigate the tannin content and the present study was an attempt towards that.

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