Determination of Phenol in Locally Grown Fruits and Vegetable by Spectrophotometric Method

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Summary: Spectrophotometric method for the determination of phenol in the sample of locally grown fruits apple, pear, sweet orange and vegetable radish of Quetta, Hyderabad and Nawabshah are described. Juices from these fruits and vegetable were squeezed, filtered and decolorized with charcoal. The antipyrine dye formed by reaction between phenol and 4-aminoantipyrine was analyzed. The calibration graphs were prepared in the range of 0.5 to 4 ppm of phenol. Phenol in apple, pear and sweet orange was found to be in the range of 1–1.2 ppm and in radish was found to be 0.5 ppm. Possible sources of organic pollutant were pointed out and were discussed. Limit of detection of the method was investigated and was found to be 0.2μg/ml.

Introduction

Phenolic compounds are widely distributed in plant kingdom and are important in fruits because they are responsible for the color and flavor of the fresh fruit and processed products [1]. They are particularly important in enology and contribute to the organoleptic nature of wines (flavors, astringency, hardness). They are also important in chemo- taxonomy and plant pathology.

Phenolic compounds have been implicated in host resistance to pathogens [2]. Pentachlorophenol is widely used in agriculture and industry as a bactericide, herbicide, mollusucicide, algicide, and insecticide(e.g against wood damaging insects) [3]. Various chlorophenols and chlorinated p-benzoquinones are formed by the reaction between phenol and chlorine including 2,4- dichlorophenol, 2,6-dichlorophenol, 2,4,6-trichlorophenol. [3-10] 2,4-dichlorophenol is suspected to have endocrine-disrupting effects [11] and 2,4,6-trichlorophenol is possibly carcinogenic to humans [12]. Moreover chlorophenol impart a bad test and odour to the fruit and vegetable [13]. Pentachlorophenol in soil is important because of its other applications especially in wood protection and the resulting release in to the environment. Several publications have reported conversion and degradation in soil and a number of conversion products have been identified [14-18].

Phenolic compounds of many fruits, such as apples, pears and grapes have been analyzed [19-21]. The Kola nut is a tropical tree crop which is important for its tonic and simulating effect, was also analyzed for its phenolic compounds. For the determination of phenol various methods are used [1]. Gas Chromatography and Liquid Chromatography have advantages, that phenol and its derivative can be simultaneously determined. Phenol is also determined by spectrophotometry [22]. Iodine monobromide was used for the iodination of phenol and its derivatives, then Iodinated phenols were determined by u.v.and visible spectrophotometer [23,24]. A number of spectrophotometric methods have been utilized for the determination of phenols [25-29]. Most of these methods are modification of spectrophotometric method introduced by Isoshi *et al* [30].

As most of the methods mentioned above need expensive instruments and lengthy procedure. Therefore there is a need for simple sensitive and economical methods. In this paper well known colorimetric method using the reaction between phenol and 4-aminoantipyrine to form a complex was used, which was determining by spectrophotometer.

Result and Discussion

Food adulteration and contamination has increased enormously as a result of human activity. In view of this situation, it was thought essential to determine phenol in locally grown fruits and vegetable in Quetta, Hyderabad and Nawab Shah using canal water and also in some area mixed to contaminated water. Fruits, vegetable along with their location of collection and the type of water used for irrigation is given in Table-1. Phenolic

Table-1: Fruits & vegetables source of water sample location.

S. No.	Sample	Type of water	Location
1.	Apple	Canal water	Quetta
2.	-do-	Canal water	Glustan
3.	-do-	Canal water	Chaman
4.	-do-	Canal water	Ziarat
5.	Pear	Canal water	Nawabshah & Hyderabad
6.	Sweet, Orange	Canal water	Nawabshah
7.	Radish	Canal water	Nawabshah & Hyderabad

compounds of many fruits, such as apple, grapes have been analyzed. [18-21]. Juices were collected from the apple, pear, sweet orange and radish and were analyzed by double beam U.V and visible spectrophotometer. Phenol content of each sample is given in Table-2. It is evident from the result that concentration of phenol was found comparatively higher in Quetta and Gulistan areas as compared to Chaman. Concentration of Phenol in Quetta, Gulistan apples samples was found to be in range of (1-1.2 µg/ml), while Chaman and Ziarat phenol concentration was detected (1 µg/ml). It is quit possible that this difference in concentration of phenol may be due to irrigated water in Quetta and Gulistan cities are contaminated with sewerage or may be by the industrial effluents, while the samples of Chaman city were slightly affected by the effluents. Secondly pesticide, insecticide material from soil and spray contaminates caused to elevate phenols level.

Table-2:Concentration of Phenol in (µg/ml) and fruit and vegetable grown on canal water.

S.No.	Sample	Concentration in (µg/ml)	Absorbance
1.	Apple-Q	1.2	0.1908
2.	Apple-G	1.1	0.1726
3.	Apple-C	1.0	0.1632
4.	Apple-Z	1.0	0.1467
5.	Pear-N	1.0	0.1602
6.	Sweat–orange N,H	1.2	0.1875
7.	Radish- N,H	0.5	0.0715

The detection level of phenol in pear, sweat orange and radishes grown on canal sides of Hyderabad and Nawab Shah are shown in Table-2. Phenol concentration in pear detected is (1µg/ml) and in sweat orange is (1.2 µg/ml), very small amount of phenol was found in radish sample (0.5 µg/ml). Generally low level of phenol concentration was found in the radish sample, while significantly higher concentration of phenol was detected in sweat orange grown in Hyderabad and Nawab Shah. This higher concentration of phenol may be due to irrigated water are contaminated with sewerage water. From the results it is evident that the concentration of phenol was significantly high in an apple and sweat orange samples. However the concentration of phenol in pear and radish was almost the same in these fruits and vegetable irrigated with canal water.

Table-3: Effect of concentration on absorbance using 4-aminoantipyrin method.

S.No.	Concentration in (µg/ml)	Absorbance
1	0.5	0.0793
2	1	0.1521
3	2	0.3054
4	3	0.4553
5	4	0.6096

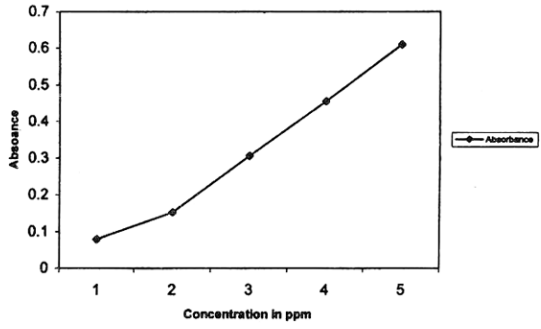

Fig 1: Effect of phenol concentration on absorbance behavior using 4-aminoantipyrine method.

Calibration curve of phenol was prepared by varying the amount of phenol in the range of 0.5-4ppm is shown in Table-3 and Fig. 1. From the calibration curve it was found that with the increase in concentration of phenol the complexation would also increase and as a result increase in the absorbance is observed. It is clear from the standard curve, that absorbance is directly proportional to the phenol concentration. Phenol concentration up to the 0.2 µg/ml could be detected by this method as shown in Table-4 and Fig. 2. This method was applied to estimate limit of detection in fruits and vegetables samples. This method is very simple, reliable and less time consuming which allow analyzing much more sample and can be used as routine detection technique.

Experimental

Apparatus

Perkin-Elmer Model Lambda 4C double beam U.V. and visible spectrophotometer was used for the

Table 4: Limit of detection of phenol based on 4-aminoantiyprine complex by indirect method.

S.No.	Concentration in (µg/ml)	Absorbance
1	0.0	-
2	0.1	-
3	0.2	0.0382
4	0.5	0.0803
5	1	0.1498
6	2	0.2904
7	3	0.4418

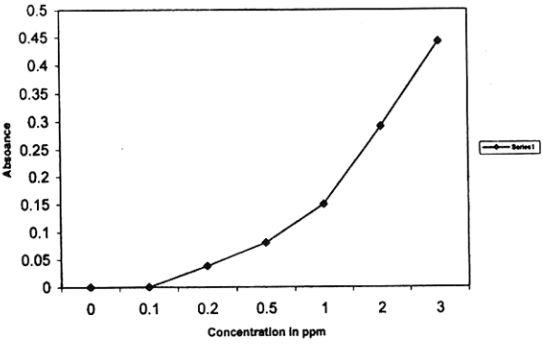

Fig 2: Limit of detection of phenol based on 4-aminoantipyrine method.

measurement of absorbance. The pH of solution was measure with Orion Research Model No. 701A/ digital Ionolyzer.

Reagents

4-aminoantipyrine of analytical grad (Fluka), Potassium Ferricyanide (BDH), Ammonia solution (Merk), Ammonium chloride (BDH), Phenol Analytical grade (BDH).

Water was purified by redistillation of distilled water in the presence of potassium permanganate. 2% 4-aminoantipyrine solution was prepared by dissolving 2.0g 4-aminoantipyrine in appropriate amount of distilled water in 100 ml volumetric flask. 4% potassium ferricynaide was prepared weakly by dissolving 4g amount of potassium ferricyanide in 100 ml distilled water. For the preparation of Buffer solution of i.e. pH=10 was prepared by adding 142 ml concentrated ammonia solution (sp. gr. 0.88-0.90) to 17.5g AR ammonium chloride and diluting to 250 ml with de-ionized water.

To prepare 1000 mg/dm^3 phenol stock standard solution, 0.1g phenol was dissolved in 100 ml of water using volumetric flask. 10 ml of 100 mg/dm^3 standard phenol solution was diluted to 100 ml with distilled water to form 100 ppm.

Procedure

3 ml of phenol solution from 100 ppm stock solution was taken in a 100 ml pyrex volumetric flask. To this 1ml of ammonia buffer solution, pH=10 and 2 ml. of 2%, 4-aminoantipyrine solution and 6 ml of 4% potassium ferricyanide solution were added in that order and diluted up to 100 ml with distilled water. The solution was checked for complex formation using U.V. and Visible spectrophotometer. For wavelength optimization studies of the resultant mixture, the solutions were subjected to variable wavelength studies in the range of 350-650 nm. The absorbance of these solutions was noted at the optimum wavelength using blank solution as reference.

Preparation of standard curve

Working standard solutions were prepared in the range of 1, 2, 3, 4, and 5ppm. by taking 1 , 2 , 3, 4 and 5ml. of 100 ppm phenol . And to these solution 1 ml of pH=10 buffer solution. 2 ml of 2 %, 4-aminoantipyurine solution, and 6 ml of 4 % potassium ferricyanide solution were added to each flask and diluted each up to 100 ml with distilled water. A blank was prepared in the same way as the working standard except the addition of phenol solution. The absorbance of these solutions was noted at 510 nm. Standard curve was prepared by plotting absorbance against concentration.

Determination of phenol in fruit and vegetable

Sample collection

A variety of fruit and vegetable sample irrigated by canal and sewerage water, were collected from the selected area of different cities (Table 1) The sample were washed with distilled water to remove debris and soil particles before proper storage in the polyethylene bags.

Extraction of juices

For extraction of juices apple, sweet orange, pear and radish were cut in to slices. Juices from these fruits and vegetable wee squeezed, filtered and decolorized with charcoal. After some time again filtered it and obtained decolorized juices.

Sample preparation

20 ml.of decolorized juices of apples, pear sweet orange and radish were taken in a volumetric flask and added 1 ml of pH=10 ammonia buffer solution to it. Then added 2 ml of 2% 4-aminoantipyrine solution and 6 ml of 4% potassium ferricyanide solution to it and diluted each up to 100 ml with distilled water. A blank was prepared in the same way as the sample except the addition of phenol solution

Conclusions

The Spectrophotometric method based on complexation of phenol could be applied for phenol determination in various samples of fruits and vegetable. The information gained from these measurements will help in establishing a baseline level of toxicity of fruits and vegetables of various cities. This method is simple, quicker, economical as well as sensitive and does not need expensive instrumentation.

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Dependence of pH on Fe(III)-Tannic Acid Complexation

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Summary: Complex formation between Fe(III) and tannic acid basically depends upon pH. Tannic acid is a catecholate type siderophore which has 8 gallic acid groups, it can bind 1-4 Fe(III) depending upon pH as well as ligand concentration. They form highly stable colored complexes with Fe(III) at pH range 4 -10. The experimental results of pH titrations were treated by program "Best". A specie distribution of the complexes like ML, ML_2, M_2L and M_4L were also determined for Fe(III)-tannic acid complexes at room temperature. These potentiometric results are comparable with spectrophotometric data.

Introduction

Tannic acid is a large molecule containing 8 groups of 1,2,3-trihydroxybenzoic acid (gallic acid) having molecular mass 1701.23. It enables to chelate more than one metals at a time (simultaneously). The stoichiometry and stability of the complexes may be pH dependent [1-4]. It is the major constituent of our beverages and food stuffs [5]. It belongs to the hydrolysable category which decomposes in water [6]. Generally its quantity found in tea is 15-20%, but it depends on many factors such as age variations, seasonal variations, extent of processing and the region where tea is grown [7,8].

Tannic acid belongs to catecholic category like enterobactine, which form highly stable complex with Fe(III) having stability constant value 10^{52} [9]. Catechols and its derivatives form stable complexes with iron and vanadium [10-12]. These have very high affinity to the Fe(III), so they form highly stable complexes with Fe(III) having stability constants 10^{30}-10^{50} even at physiological pH [13-17].

One of the most important reasons to cause anemia is due to the fact that, maximum absorption of iron in the biological environment takes place, in the form of Fe(II), but we intake a large amount of iron by the food stuffs in the form of Fe(III).

It is suggested that tannins are responsible for the interference of absorption of iron in the body [18,19]. Therefore, tannic acid in any form of beverages either hot or cold, consumed by the human bodies resulted in deficiency of iron. The study of complexation of this multidentate ligand with Fe(III) seems to be valuable and interesting. The change of pH may have remarkable effect on chelation.

Results and Discussion

Ligand has capability to bind 1-4 metal ions due to several binding sites A, B, C and D. Where A and B are preferable as compared to C and D sites. At low pH, since ligand is in protonated form, A and B positions form only two bonds with each metal (act as bidentate) and remaining coordination sites of the metals might be satisfied with water molecules. However at high pH values the ligand is completely free from protons therefore, position A and B act as hexadentate whereas position C and D will be act as bidentate for third and forth metal. These all spectrophotometric results have also been discussed in detail previously [20] which are compareable with potentiometric results.

The pH dependence of the complex formation is determined by the potentiometric study, in this study pH titrations are performed and noted the change in pH after the addition of standard NaOH solution. These titrations are performed in two ways; one with complex solution with NaOH solution and the second one with ligand solution.

In the first case three sets are titrated in the first one when ML ratio was 1:1 or metal and ligand taken in equal volume then curve of the complex showed that two depression at low and high pH because of the effect of deprotonation and complexation. As pH increases ligand becomes more negative and more and more binding sites are available for metal ions. Due to the presence of equal amount of ligand, at low pH only ML specie is possible while at high pH mixed specie of M_2L with ML_2 may form (Figure 1). In the second set, when ligand solution is four times as metal ion solution then only ML specie is possible because ligand is in

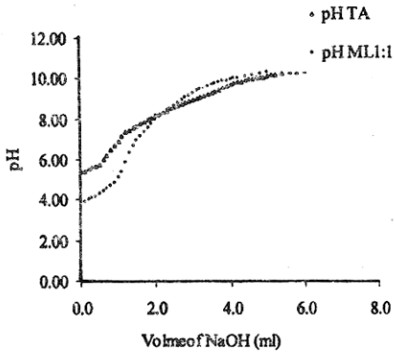

Fig. 1: Plot for pH titration of Fe(III)-tannic acid Fe(III) solution and tannic acid solution with equal volume.

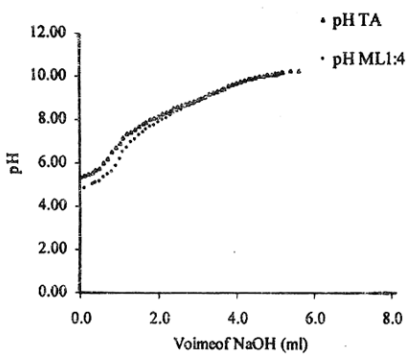

Fig. 2: Plot for pH titration of Fe(III)-tannic acid aannic acid solution four times as Fe(III) solution.

very large amount. All binding sites are available for metal but metal ion is not in excess so only ML species is formed (Figure 2). In the third set when metal ion is taken four times as ligand solution, curve of complex showed that a clear change between the pH range 4-10. As the pH increases co-ordination sites of ligand increase and because of the large amount of metal ion there is only possibility of M_4L specie (Figure 3).

Another titration in which ligand is treated with metal ion solution, the curve shows the same results at three points by three depressions. When ligand is in high amount only ML specie may be formed with increasing metal ion concentration M_2L specie and with excess of metal ion M_4L specie may form (Figure 4). These results are very much agreed with the above assumptions.

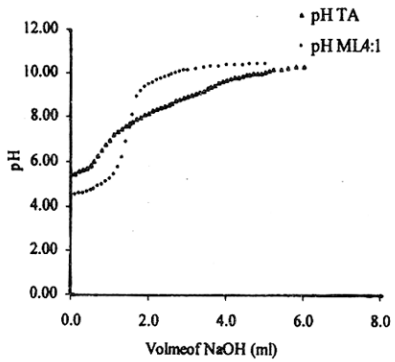

Fig. 3: Plot for pH titration of Fe(III)-tannic acid Fe(III) solution four times as tannic acid solution.

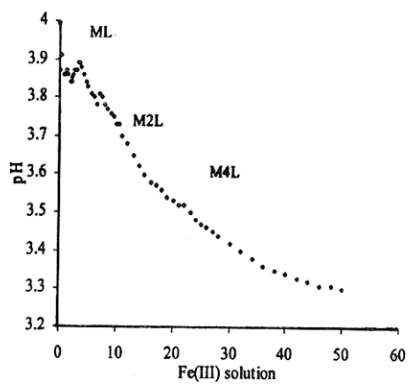

Fig. 4: Plot for pH titration of Fe(III) with ligand solution.

Species distribution is also determined from the "Best" for Fe(III)-tannic acid complex at room temperature [1]. Species distribution graphs are drawn between fraction of metal and complexes versus pH for the low ligand concentration as well as for the high ligand concentration. These graphs exhibit that concentration of uncomplexed metal ion has gradually decreased and amount of complex has increased (Figure 5-6).

Experimental

Analytical grade (AR) reagents are used without any further purification. Double distilled deionized water is used in working solution and preparation of all solution of reagents and buffers, this double distillation was taken by the deionizer (i.e. Amberlite resin RA-401 from BDH chemicals)

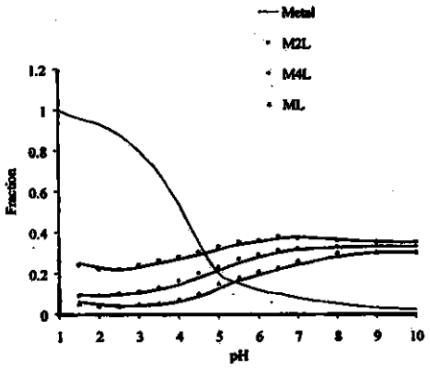

Fig. 5: Plot for specie distribution at different pH low ligand concentration.

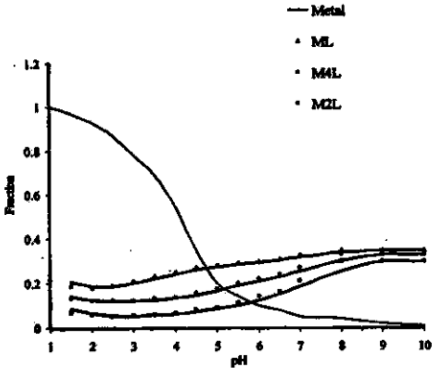

Fig. 6: Plot for specie distribution at different pH high ligand concentration.

in order to make it free of cations (Check by the conductivity method).

For pH titration, CO_2 free water is prepared by boiling redistilled and deionized water for 10 minutes and then cooling it in air tight flask. A 0.050M solution of Potassium Hydrogen Phthalate which has the pH vale 4.01 at 25°C is used to calibrate pH meter.

pH titrations are done using pH meter with double walled titration cell kept on a magnetic stirrer (ORION Research Analog pH meter/model SA 920A). Standard solutions of NaOH and $Fe(NO_3)_3 9H_2O$ are used. Five sets of titration are performed, in the first set only tannic acid solution (0.00010M) is taken in a beaker, then aliquotes of standard NaOH solution (0.010M) is added with the help of pipetman model Gilson made in France and the pH variation is measured after each addition. Then following the same procedure three sets of complex solutions are titrated against standard NaOH solution (0.010M). Equimolar solutions (0.00010M) of $Fe(NO_3)_3.9H_2O$ and tannic acid are used. In the second set equal volume of each solution is mixed and then titrated. Then for third set tannic acid solution is taken four times as iron solution while in the fourth set iron solution is taken four times as tannic acid solution. In the fifth set tannic acid solution (0.00010M) is titrated against iron solution (0.0010M) in the same manner.

Conclusions

When equal volumes of Fe(III) and tannic acid are mixed at low pH ML specie and at high pH mixed specie is formed due to the effect of deprotonation and complexation. In the presence of high amount of tannic acid only ML specie is formed while with high metal concentration only M_4L specie is formed due to maximum deprotonation. When only tannic acid is treated with metal solution same behavior of ligand is observed. With continuous addition of metal causes decrease in pH as well as binding sites of ligand are increased so stoichiometry altered from ML to M_4L.

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