# Characterization and Statistical Analysis of Bioactive Compounds from Fruit Wastes

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**Abstract:**

Fruit consumption is no longer merely a result of taste and personal preference, but has become a concern of health due to the vital fruit nutrients content. In addition to essential nutrients, most fruits feature considerable amounts of micronutrients, such as minerals, fibers, vitamins and secondary phytochemical compounds. Increasing evidence shows the importance of these micronutrients for human health .Diets rich in phytochemicals, such as carotenoids and phenolic compounds, have been associated with a reduced risk of diseases such as certain types of cancer, inflammation, cardiovascular, cataracts, macular degeneration and neurodegenerative diseases.The preesnt study aims at extracting the bioactive components of fruit wastes like pecton, antimicrobial proteins and polyphenols.The satistical analysis of the compounds in respect to their correlation coefficients have been studied.'



## 1. INTRODUCTION:

Due to the high consumption and industrial processing of the edible parts of fruit, fruit wastes such as citrus fruit skins, pineapple residues, sugarcane bagasse and other fruit residues (principally peels and seeds) are generated in large quantities in big cities. Fruit waste has become one of the main sources of municipal solid wastes (MSW), which have been an increasingly tough environmental issue. At present, the two main techniques to dispose MSW are

landfill and incineration. However, inappropriate management of landfill will result in emissions of methane and carbon dioxide **(Qdias *et al.*, 2010),** and incineration involves the subsequent formation and releases of pollutants and secondary wastes such as dioxins, furans, acid gases as well as particulates **(Buekens *et al.*, 1998**), which pose serious environmental and health risks. For these reasons, there is an urgent need to seek resource and value-added use for fruit wastes. In fact, inexpensive and readily available use of agri-food industry waste is highly cost-effective and minimizes environmental impact. One of the most beneficial approaches is to recover the bioactive constituents, especially the phenolic compounds, making full use of them in the food, pharmaceutical as well as cosmetics industry **(Makris *et al.*, 2007)**. Thus, utilization of the fruit wastes as sources of bioactive compounds may be of considerable economic benefits and has become increasingly attractive.



Epidemiological studies indicated that the frequent consumption of fruits is associated with a lower risk of chronic diseases **(Nowson *et al.*, 2007 , Bae *et al.*, 2008).** Natural antioxidants in fruits and vegetables, such as vitamins and polyphenols, are considered to be responsible for these health benefits **(Ruxton *et al.*, 2006 Saura-Calixto *et al.*, 2006).** Due to the potential health risks of some synthetic antioxidants **(Ito *et al.*, 1986, Safer *et al.*, 1999**), increasing attention is being paid to identify natural and possibly more economic and effective antioxidants. Phenolic compounds are one of the most important categories of natural antioxidants of interest, and much evidence is derived on the antioxidant potency as well as their prevention of diseases **(Fresco *et al.*, 2006, Cheng *et al.*, 2007, Scalbert *et al.*, 2005)**. Yet, in recent studies, the antioxidant potency and the content of phenolic compounds were found to be high in the peel and seed of some **fruits (Ajila *et al.*, 2007, Kunradi *et al.*, 2009, Okonogi *et al.*, 2007**),

indicating that fruit residues have the potential to be utilized as a resource of bioactive compounds, such as natural antioxidants.

The current study aims at reducing the contaminant load through formulation of value added products like pectin. The waste obtained in the pectin extraction process was further utilized to extract heat stable proteins (HSP) and the antimicrobial activity of the protein was studied.The residual waste was also analyzed for antioxidant activity and total phenolic compound analysis. The current study aims at the statistical analysis of the value added and bioactive products of the different fruit wastes.

## 2. MATERIALS AND METHODS:

### Extraction of Bioactive compounds from Fruit Wastes:

Fruit peels from *Malus domestica* (cv. 'Apple') *Musa acuminata* (cv. 'Cavendish') and *Citrus limetta* (cv. 'Sweet lime'), rind of *Citrullus lanatus* (watermelon) and soggy whole fruits of *Solanum lycopersicum* (cv. 'Roma tomato') and *Psidium guajava* (cv. 'Red Indian') were mixed separately with de-ionized water in the ratio 1: 1.5 and macerated, pH was adjusted using lemon juice to 2-2.5. It was autoclaved for 15 min and filtered, the filtrate yields pectin **(Schemin *et al*., 2005**). The gel grade and methoxyl content of the extracted pectin was studied.The unused residue was sun dried, utilized for protein extraction and was tested for its antimicrobial and antioxidant properties.

### Estimation of the Proteins and Determination of its antimicrobial activity:

The protein was extracted from the residue using the extraction buffer (1M Tris HCl pH 7.6, EDTA 0.5 M pH 8.0, ascorbic acid and β mercaptoethanol. Mixture was kept for 20 minutes at

4°C on a magnetic stirrer. It was centrifuged at 10,000 rpm at 4°C, supernatant containing TSP was heated at 70°C for 10 minutes. It was centrifuged at 10,000 rpm at 4°C to obtain HSP. The amount of protein was quantified spectrophotometrically (280 nm). The HSP obtained from various fruit wastes was utilized to assess the antimicrobial activity.

**Antimicrobial activity of the HSP**

The well diffusion method **(Shobha and Kale , 2008)** was followed to assess the antimicrobial activity of the HSP. Cultures of *Escherichia coli* and *Pseudomonas aeruginosa* were swabbed uniformly on nutrient agar plates. The concentrations tested included 50, 75 and 100% in 100ul of the original protein levels in the sample.sterile distilled water was used as control in all the plates.Evaluation of the antifungal activity against *Fusarium oxysporum* (on potato dextrose agar) was carried similarly. All the antimicrobial assays were carried in triplicates.



**Estimation of the antioxidant activity and total phenolic content:**

The antioxidant activity of the fruit wastes was assayed by measuring the Ferric Reducing Antioxidant Power(FRAP) (**Benzie and Strain, 1996)**. The residues of the six different fruit wastes were extracted using 50% methanol at room temperature and centrifuged at 10,000 rpm. The supernatants were filtered after washing with equal volumes of petroleum ether to remove the oil content.The FRAP reagent (10mM/L TPTZ 2.4.6 tripyridyl- s- triazine)in 40mmol/L HCl plus 20mM/L $FeCl_3$ and 0.3mmol/L actetate buffer, pH 3.6) was awrmed at $37^0$C and mixed with methanolic extract. After incubating the mixture for 10 minutes at $37^0$C the reactant was measured at 593nm.

Total phenolics were determined colorimetrically **(Velioglu *et al.* 1998**) with slight modifications. The methanolic extracts of the residues obtained from the different fruit wastes were mixed individually with F-C reagent (diluted 10 fold with distilled water) and incubated at 22°C, for 5 min. Sodium bicarbonate solution (60 g/L) was added and after 90 min at 22°C, absorbance was measured at 725 nm. Total phenolics were quantified by calibration curve obtained from measuring the absorbance of a known concentration of gallic acid. The total antioxidant assay and the total phenolic compound analysis were done in triplicates and the mean values were considered for statistical analyses.



**Statistical Analysis of the Study:**

Data obtained from the 6 fruit samples was entered into SAS 9.3 (SAS Institute Inc., NC, USA) and analyzed. The mean antioxidant, antibacterial, antifungal contents of the samples at the 50% zone of inhibition were computed. Additionally, the mean phenolic content, dry and wet pectin weights were calculated for the samples.

In the next step, correlation was performed among the above 6 variables to examine if there was any relationship across the variables. The correlation co-efficient, which is normally denoted by *r*, provides a measure of association of two variables in a sample. Three important properties of *r* includes, it takes on values that range from -1 to +1, It is dimensionless, i.e., the value of *r* is independent of the units of measurement of the variables, It can be positive, negative, or zero.

A value of $r$ = +1.0 denotes a perfect linear relationship between the variables; that means, if the value of one variable increases, the value of the other variables increases in an exact linear fashion. Similarly, a value of $r$ = -1.0 indicates a perfect negative relationship. Generally, values of $r$ between 0 and 0.3 (0 and -0.3) indicate a weak positive(negative) relationship; values between 0.3 and 0.8 (0 and -0.8) indicate a moderate positive (negative) relationship, and values greater than 0.8 (-0.8) denote a strong positive (negative) relationship.

In addition to that, the correlation co-efficient statistic has some properties which closely relate it to linear regression, and gives $r$ an alternative interpretation in terms of straight-line relationship. A different way of looking at linear regression is to consider two random variables X and Y having bivariate normal distributions. Aunivariate normal distribution is described by a density function that is a bell-shaped curve when plotted in two dimensions. Similarly, the bivariate normal distribution can be described by a joint density function whose plot appears as a bell-shaped curve in three dimensions.In other words, the distribution of Y for fixed X is univariate-normal. Under the assumption of bivariate normal distribution, the value of $r^2$can be expressed as the percent of variation in Y accounted for by X. Thus, the statistic $r^2$also provides a measure of the strength of linear relationship between X and Y.

In the current study, the bivariate correlation of the 6 variables was calculated. The Pearson correlation co-efficient along with the p-value was reported for each association.

**RESULTS:**

**Extraction of pectin:**

The pectin content of the fruit wastes was ranging from 3-14% with maximum yield of pectin from *Citrus limetta* and the minimum from *Malus domestica* peels **Table 1.** The results obtained in the present study suggest presence of high protein content in fruit wastes. The concentrations of TSP and HSP extracted from the residual waste are given in **Table 2.**It was found that TSP and HSP concentrations in *S. lycopersicum* waste was highest (2.17 mg/mL and 1.48 mg/mL) and lowest in *Musa* sp. waste (0.93 mg/mL and 0.90 mg/mL). The gel grade of the extracted pectin was in the range of 150-200 and methoxyl content was in the range of 4-14%.

**Antimicrobial activity of HSP**

The current study assess the antimicrobial activity of the HSP isolated from the fruit waste. In the present study the undiluted HSP extracted from *C. limetta* has shown larger zone of suppression against *E. coli* (2.73 cm) (**Table 3**). The undiluted HSP (100%) from *Musa acuminate* and *C .limetta* peels showed the highest suppression of *Pseudomonas aeruginosa*. (3.33 cm). HSP from peels of *Musa acuminata* showed good antimicrobial effect, although it had the least concentration of HSP (**Tables 3**).

Therefore, the HSPs extracted from various fruit wastes showed similar antimicrobial properties against the test organisms, irrespective of the source and concentration. They were efficient even at low concentrations and the antimicrobial effect did not increase much at higher concentrations. The results clearly indicate that water soluble, temperature tolerant (non enzymic) proteins have an important role as antimicrobials.

HSP from *C. limetta* waste (100, 75 and 50%) showed highest suppression of *Fusarium oxysporum*, least was by HSP from *C. lanatus* (**Table 4**).It ascertains the presence of

considerable levels of antifungal activity in various other fruit wastes. The antifungal activity of the HSPs was observed to be concentration dependant unlike the antibacterial activity i.e. its activity increased with increasing concentration (**Table 4**).

**Total phenolic compound and related antioxidant activity in the residual fruit waste**

The growing interest in the substitution of synthetic food antioxidants by natural ones has fostered research on fruit and vegetable sources and the screening of raw materials for identifying new antioxidants. The total phenolic compounds in the residual waste were calculated from the Gallic acid (GA) standard graph. The highest phenolic content was found in *Psidium* sp. (0.63 g GA/mL) and lowest phenolic content was found in *S. lycopersicum* residual waste (0.09 g GA/mL) (**Table 5**).



The highest FRAP value was obtained for the extract from *Psidium guajava*. (8.75 mmol $Fe^{2+}$/mL), followed by *Musa acuminata*. (8.56 mmol $Fe^{2+}$/mL) and least for *S.lycopersicum* residual waste (2.95 mmol $Fe^{2+}$/mL) (**Table** 5)

**Statistical Analysis of the Study:**

In the current study, the bivariate correlation of the 6 variables was calculated. The Pearson correlation co-efficient along with the p-value was reported for each association.

Table 6 presents the descriptive statistics of the samples. Analysis of data found that the mean antioxidant content of the sample was 6.89±2.34 $Fe^{2+}$/ ml, mean phenolic content was 0.29±0.21 g GA/ ml, mean antibacterial content was 2.26±0.13 cm, mean antifungal content was 1.17±0.46 cm, mean pectin wet weight 586.81±572.72gms, and mean pectin dry weight was 32.36±30.99gms.

Table 7 presents the results of correlation analysis. The study found that, antioxidant and phenolic contents were highly positively correlated; r = 0.81. Antibacterial and antioxidant properties were also found to be positively correlated (r=0.45). High correlations were also found between phenol & antibacterial contents, and phenol & pectin contents.

A study by **Hajimahmoodi *et al.***, in 2008 found that, 97% of the variance in antioxidant activity was explained by phenolic activity.Therefore we computed the $r^2$ statistic between phenolic and antioxidant contents of the sample to see the extent of variation in antioxidant activity that could be accounted for by the phenolic activity. Our study found that, the corresponding $r^2$ for phenolic content and antioxidant activity was $(0.81)^2$ = 65%. This meant, 65% of the variance in antioxidant activity could be explained by phenolic activity in our sample. However, since the calculation of $r^2$ was based on bivariate normal assumption, and the optimal sample size for the study was low (n<30), the study findings may be sensitive to sample size. Therefore, caution should be exercised while interpreting the study findings. Consequently, larger sample sizes are required to validate our study findings.



**Discussions:**

The potential of fruit and vegetable waste is not only limited to the production of value added products like pectin but could also be utilized in generating bioactive compounds like antimicrobials and antioxidants and can eventually bring down the contaminant load in the environment.



**Table 1. Pectin content from per kg of Fruit wastes**

| S.No | Fruit waste | WetWeight (in gms) | Dry Weight (in gms) | Percentage of pectin E |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| 1. | *Solanum lycopersicum* | 105.00 | 07.50 | 7.14 |
| 2. | *Citrus limetta* | 182.26 | 26.79 | 14.69 |
| 3. | *Musa acuminata* | 1409.33 | 78.60 | 5.57 |
| 4. | *Citrullus lanatus* | 454.00 | 15.50 | 3.41 |
| 5. | *Psidium guajava* | 1200.3 | 62.30 | 5.19 |
| 6. | *Malus domestica* | 170 | 3.445 | 2.07 |

**Table 2: Estimation of HSP and TSP from Fruit Wastes**

| Fruit Wastes | TSP concentration mg/ ml | HSP concentration in mg/ml |
|---|---|---|
| *Musa acuminata* | 0.93 | 0.90 |
| *Citrus limetta* | 1.73 | 1.38 |
| *Citrullus lanatus* | 1.84 | 1.40 |
| *Solanum lycopersicum* | 2.17 | 1.48 |
| *Psidium guajava* | 1.43 | 1.40 |
| *Malus domestica* | 1.76 | 1.37 |



**Table 3** Antibacterial activity of HSP extracted from different fruit wastes against *E. coli and Pseudomonas aeruginosa*. (n=3).

| **Fruit waste** | **Zone of inhibition Mean ± S.D (cm)** | | | |
|---|---|---|---|---|
| | ***Escherichia coli*** | | ***Pseudomonas aeurgimosa*** | |
| | **HSP concentration** | | | |
| | **100%** | **50%** | **100%** | **50%** |
| *Musa* sp. | 2.93 ± 0.11 | 2.30 ± 0.10 | 3.33 ± 0.47 | 2.53 ± 0.25 |
| *Citrus limetta* | 2.73 ± 0.11 | 2.33 ± 0.06 | 3.33 ± 0.28 | 2.70 ± 0.17 |
| *Citrullus lanatus* | 2.73 ± 0.11 | 2.30 ± 0.17 | 2.80 ± 0.53 | 2.40 ± 0.45 |
| *Solanum lycopersicum* | 3.13 ± 0.32 | 2.26 ± 0.21 | 2.83 ± 0.21 | 2.43 ± 0.30 |

| | | | | |
|---|---|---|---|---|
| *Psidium* sp. | 2.90 ± 0.10 | 2.36 ± 0.23 | 3.26 ± 0.40 | 2.70 ± 0.17 |

HSP = Heat Stable Protein

**Table 4** Antifungal activity of HSP against *Fusarium oxysporum* (n=3)

| **Fruit waste** | **Zone of inhibition (cm) Mean ± S.D** | | |
|---|---|---|---|
| | **HSP concentration** | | |
| | **100%** | **75%** | **50%** |
| *Musa acuminata*. | 2.26 ± 0.31 | 1.86 ± 0.40 | 1.33 ± 0.35 |
| *Citrus limetta* | 2.70 ± 0.17 | 2.10 ± 0.17 | 1.60 ± 0.17 |
| *Citrullus lanatus* | 1.76 ± 0.05 | 1.46 ± 0.11 | 1.10 ± 0.17 |
| *Solanum lycopersicum* | 2.00 ± 0.20 | 1.53 ± 0.15 | 1.16 ± 0.15 |
| *Psidium guajava* | 1.93 ± 0.11 | 1.03 ± 0.25 | 0.33 ± 0.28 |
| *Malus domestica* | ± | ± | ± |



## Table 5: Antioxidant Activity and Polyphenol Content of the Residual Fruit Wastes

| Fruit Wastes | FRAP value mmol $Fe^{2+}$/1 ml | Total Phenol g GA/ 1ml |
|---|---|---|
| *Musa acuminata* | 8.563 | 0.45 |
| *Citrus limetta* | 7.485 | 0.31 |
| *Citrullus lanatus* | 6.794 | 0.13 |

| | | |
|---|---|---|
| *Solanum lycopersicum* | 2.953 | 0.08 |
| *Psidium guajava* | 8.745 | 0.63 |
| *Malus domestica* | 5.56 | 0.15 |

**Table 6: Descriptive statistics of the sample**

| Sample Statistics | | | | | | |
|---|---|---|---|---|---|---|
| **Variable** | **N** | **Mean** | **Std Dev** | **Sum** | **Minimum** | **Maximum** |
| Antioxidant | 6 | 6.67000 | 2.16217 | 40.02000 | 2.95000 | 8.74000 |
| Phenol | 6 | 0.29167 | 0.21151 | 1.75000 | 0.09000 | 0.63000 |
| Antibacterial | 6 | 2.26000 | 0.12696 | 13.56000 | 2.01000 | 2.36000 |
| Antifungal | 6 | 1.17333 | 0.45684 | 7.04000 | 0.33000 | 1.60000 |
| Wet Pectin | 6 | 586.81500 | 572.72677 | 3521 | 105.00000 | 1409 |
| Dry Pectin | 6 | 32.35667 | 30.99559 | 194.14000 | 3.45000 | 78.60000 |
| Gel grade | 6 | 156.50000 | 35.40480 | 939.00000 | 100.00000 | 200.00000 |
| MethoxylContent | 6 | 6.36833 | 1.02644 | 38.21000 | 5.52000 | 7.89000 |



**Table 7. Correlation co-efficients among antioxidant, antibacterial, antifungal, phenol, dry pectin weight and wet pectin weights in the sample**

| Pearson Correlation Coefficients, N = 6<br>Prob> \|r\| under H0: Rho=0 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Antioxidant** | **Phenol** | **Antibacterial** | **Antifungal** | **Wet Pectin** | **Dry Pectin** | **Gel grade** | **Methoxyl** |
| | 1.00000 | 0.81986 | 0.45112 | -0.29509 | 0.75691 | 0.78973 | 0.83889 | 0.50015 |
| Antioxidant | | 0.0458 | 0.3692 | 0.5702 | 0.0815 | 0.0617 | 0.0368 | 0.3123 |
| | 0.81986 | 1.00000 | 0.52133 | -0.59783 | 0.82524 | 0.87620 | 0.74020 | 0.46938 |
| Phenol | 0.0458 | | 0.2888 | 0.2101 | 0.0431 | 0.0220 | 0.0925 | 0.3476 |
| | 0.45112 | 0.52133 | 1.00000 | -0.47515 | 0.45333 | 0.55938 | 0.34393 | 0.55632 |
| Antibacterial | 0.3692 | 0.2888 | | 0.3409 | 0.3666 | 0.2484 | 0.5045 | 0.2516 |
| | -0.29509 | -0.59783 | -0.47515 | 1.00000 | -0.52701 | -0.40728 | -0.30740 | -0.75542 |
| Antifungal | 0.5702 | 0.2101 | 0.3409 | | 0.2827 | 0.4229 | 0.5534 | 0.0824 |
| | 0.75691 | 0.82524 | 0.45333 | -0.52701 | 1.00000 | 0.95675 | 0.38454 | 0.28596 |
| Wet Pectin | 0.0815 | 0.0431 | 0.3666 | 0.2827 | | 0.0028 | 0.4516 | 0.5828 |
| Dry Pectin | 0.78973 | 0.87620 | 0.55938 | -0.40728 | 0.95675 | 1.00000 | 0.45980 | 0.21226 |

| Pearson Correlation Coefficients, N = 6<br>Prob> \|r\| under H0: Rho=0 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Antioxidant** | **Phenol** | **Antibacterial** | **Antifungal** | **Wet Pectin** | **Dry Pectin** | **Gel grade** | **Methoxyl** |
| | 0.0617 | 0.0220 | 0.2484 | 0.4229 | 0.0028 | | 0.3589 | 0.6864 |
| | 0.83889 | 0.74020 | 0.34393 | -0.30740 | 0.38454 | 0.45980 | 1.00000 | 0.63171 |
| Gel grade | 0.0368 | 0.0925 | 0.5045 | 0.5534 | 0.4516 | 0.3589 | | 0.1785 |
| | 0.50015 | 0.46938 | 0.55632 | -0.75542 | 0.28596 | 0.21226 | 0.63171 | 1.00000 |
| Methoxyl | 0.3123 | 0.3476 | 0.2516 | 0.0824 | 0.5828 | 0.6864 | 0.1785 | |



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