Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2013, 5 (1):13-23
(http://scholarsresearchlibrary.com/archive.html)

Der Pharmacia Lettre
Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Studies on protein content, protease activity, antioxidants potential, melanin composition, glucosinolate and pectin constitution with brief statistical analysis in some medicinally significant fruit peels

Saranya Chitturi*, Venkata Gopichand. Talatam and Suneetha Vuppu

School of Bio Science and Technology, VIT University, Vellore, Tamilnadu, India

---

ABSTRACT

Recently, many studies have been oriented towards improving methods and efficiency of recovery from different fruit industry wastes. The investigations carried out were mainly aimed at evaluating Pectin content, Protease activity, Protein Levels , antioxidant potential and melanin composition of air-dried medicinally significant domestic fruit peels (Gooseberry (Phyllanthus emblica), Apple (Malus domestica), Redbanana (Musa Acuminata), Jujube (Ziziphus Zizyphus), Papaya (Carica), Guava (Psidium), Avocado (Persea Americana), Watermelon (Citrullus Lamatus), Muskmelon (Cucumis Melo), Kiwi fruit (Actinidia Delicosia), Orange (Citrus Limetta), Pineapple (Ananas Comosus), Mango (Mangifera), Pomegranate (Punica Granatum), Sapota (Manilkara Zapota) and their extracts. And a brief statistical analysis of the different parameters being estimated was carried out using a software called Statistics Calculator.. In the experiments accomplished, the highest Protease activity was found in peel of Jujube (2.61%) followed by Pineapple and Papaya Peels (1.84% and 1.58% respectively). Highest concentration of protein was also found in Kiw peel (1.79%) where as the highest antioxidant concentration was observed in the peel of Gooseberry (2.39%). Also the maximum levels of melanin formation was noted in the peels of Mango peel (2.19%).Highest Glucosinolate percentage concentration was found in Guava peel (3.87%). Elevated levels of % pectin was also noted in Papaya peel (5.32%). We failed to reject the null hypothesis H_0 for the Chi-square test of independence with P value < 0.99 for different estimations made in the fruit peels to learn that the percentage concentration values for Protein, Protease, Antioxidant, Melanin, Glucosinolate and Pectin are strictly independent of each other.

Key words: Protease, Protein, Melanin, Antioxidants, Glucosinolate, Pectin

---

INTRODUCTION

The medicinal significance of fruits and their products was known to mankind since times immemorial[1]. In this regard, numerous natural medicinally important fruit peels have been evaluated for their Pectin content, Protease activity, Protein Levels , Antioxidant potential , Melanin composition Glucosinolate constitution and research outcomes have shown that having a better understanding about waste products (i.e., fruit peels) from processing of agricultural commodities could offer practical and economic ways of their better usage. Peels also essentially contribute to the total weight of the fruits and remain as the primary by-product in any of the fruit product based industry. The manipulation of food processing wastes is now becoming a very serious environmental issue. At present, these peels are mostly being discarded and are being real sources of solid wastes at large expense. It is thus significant and even essential to find the potent applications for these peels.

Fruit peels are an important source of bioactive compounds including Anti-oxidants, Proteins and Pectins and just about any further detail in their better analysis like checking their Protease activity and Melanin content will help us in using these medicinally substantial byproducts to their fullest potential or help us in establishing newer uses for these peels rather than just using them as livestock feed. The main reasons behind taking up each of these studies includes the level of importance they carry in fruit metabolism, their molecular structure, biological activity and nutritional .properties and their medicinal significance i.e., their contribution towards health giving properties which when properly capitalized on can lead us to their best possible usage[2].

Overall protease activity in fruit peels:
Protease enzymes help in break down of proteins. They aid in the process of digestion and metabolism, and also are recognized to strengthen the immune system by improving inflammation. Protease enzymes though can be used as supplements and are added to certain foods, they occur naturally in certain fruits. Therefore estimating the overall protease activity in the fruit skins and peels will help us in dealing with these wastes in a more useful way[3].

Protein levels in fruit peels:
Proteins are critical sources of nitrogen as well as sulfur and are essential dietary constituents. They are major structural components that providing mechanical support to the body known to be essence of life processes for proper growth and development of all the living beings. Protein deficiency may lead to a number of health disorders. Although fruits are not major sources of protein as compared to other dietary supplements. Knowledge regarding peels as source of protein deposits will help us in designing many economically useful projects like that of the design of a single cell protein and help in their better utilization.

Antioxidant potential of fruit peels:
Antioxidants succor oxidation processes in human body as well as in food products. They occur in almost all edible plant products forming a reliable part of nutrition. Polyphenols being the most important groups of antioxidants also have several proven anticancer properties, and they are present in fruits and vegetables. This study in particular was therefore carried out to expose the antioxidant potential of these medicinally significant fruit peels[4].

Melanin composition in fruit peels:
Browning of fruits and vegetables is a potential problem during their storage and handling which is mainly due to the oxidizing process of phenolic substrates by polyphenol oxidase. Browning mainly in fruits is a chemical reaction between catechol and oxygen whose product is Benzoquinone, a brown compound toxic in nature to bacteria. When the fruit peel suffers injury catechol released safeguards the fruit by reacting with with oxygen[5].

These studies on melanin content of different fruit peels is significant because it can help in better marketability and extended shelf-life of many fruits, it also reduces fruit wastes by better utilization of peels and fruit skins. Shelf life of these fruits can be extended by not letting oxygen come in contact with injured regions[6].

Glucosinolate content in fruit peels:
Glucosinolates which are derivatives of glucose and amino acids and are known to contain nitrogen and sulfur compounds formed as secondary metabolites mostly found in the plant varieties related to Brassiacaceae familie. The well known theory for the usefulness of their presence is that upon tissue damage plant defence mechanism becomes active to release compounds like isothiocyanate, nitrile, thiocyanate upon hydrolysis rendering it toxic to herbivores and pathogens[7]. Therefore this gucosinolate estimations carried out in different fruit peels can serve as an important parameter which can contribute to the food safety during different fruit product processing techniques.
Pectin content in fruit peels:

Pectin a polysaccharide finds extensive applications in food as well as pharmaceutical industries. It is usage ranges from being a thickening or gelling agents in the food based industry to being a antidiarrhea, detoxification and blood glucose lowering agent in the pharma industry. Pectin is embodied with a linear backbone of d-galacturonic acid units and branched regions containing neutral sugars. Based on the presence of into free or esterified carboxyl group to the galacturonic acid , pectin can be classified into high- and low- methoxyl types. Analysis of some medicinally important fruit peels for their pectin content can therefore lead to them being used as conventional sources manufacturing commercial pectin[8].

MATERIALS OF METHODS

Preparation of fruit peel extracts:
Peels of 15 medicinally significant domestic fruits available in Vellore district of South India Gooseberry (*Phyllanthus emblica)*, Apple (*Malus domestica*), Red banana (*Musa Acuminata*), Jujube (*Ziziphus Zizyphus*), Papaya (*Carica*), Guava (*Psidium*), Avocado (*Persea Americana*), Watermelon (*Citrullus Lamatus*), Muskmelon (*Cucumis Melo*), Kiwi fruit (*Actinidia Delicosia*), Orange (*Citrus Limetta*), Pineapple (*Ananas Comosus*), Mango (*Mangifera*), Pomegranate (*Punica Granatum*),Sapota (*Manilkara Zapota*) were used in the investigation. These fruits were gathered either directly from fruit juice vendors or were bought at local markets. For examination only healthy looking fruits were chosen ,and were washed then peeled and their peels were carefully separated. And also the peels obtained from the fruit juice vendors were repeeled and washed to remove any amount edible portions. The peels were air dried for about a period of 1 week and ground to a fine powder and passed through a mesh sieve[9].

These powdered samples were later extracted with distilled water at room temperature by filtering them through Whatman no.2 filter paper for removal of peel particles. In case of thicker peels the residue was re-extracted twice under the same conditions to ensure complete extraction.

Protease Estimation:
15 test tubes with 3.8ml TCA were pipetted out and to these test tubes 20ml casein solution was added. 0.2ml of culture filterate of each fruit peel extract was added into its respective test tube and marked and incubated at 37°C for 1 hr. After this the test tubes were immediately transferred to an ice box for 10min to stop the reaction taking place.The absorbance was read using a spectrophotometer at 280nm.The percentage concentration of protease was estimated .

Protein Estimation:
The Protein content in the fruit peels was estimated by Lowry's method using a standard curve of Bovine Serum Albumin (BSA) solution (20-100 Mg/ml) and O.D. at wavelength of 660nm using double beam UV-Visible spectrophotometer . And the percentage concentration of protein was estimated using standard graph.

Antioxidant Estimation:
Reducing power assay method was employed and different fruit extracts (200µg/ml) were prepared and were mixed with phosphate buffer and potassium ferri cyanide. The mixture was incubated at 50ºC for 20 minutes. To this mixture, 2.5ml of 10% trichloro acetic acid (TCA) was added and the mixtures were centrifuged at 3000 rpm for 10min. The upper layer of the solutions were mixed with distilled water and ferric chloride and the absorbance was measured at 700nm and percentage concentration of antioxidants was determined.

Melanin Estimation:
For Master solution:
0.5g of peel extracts were taken into 15 test tubes and 3ml of 1N HCl was added to each.This was followed by the addition of 2ml of 1N NaOH to each of these test tubes. These test tubes were later stored in waterbath at 72°C for 10 minutes.

1ml of each of the master solutions were taken into 15 test tubes. 5ml of 1N HCl was added to each of these test tubes followed by the addition of 2ml 1N NaOH and these test tubes were then placed in a water bath at 72°C for 10 minutes. 1ml of 1N HCl was added to these test tubes after they were taken out of the water bath followed by the addition of 1N NaOH to each of them.Again these 15 test tubes were kept in a waterbath at 72°C for 10 minutes and were again cooled by placing in an ice box. And the absorbance was read at 700 nm and percentage concentration was determined.

Glucosinolate Estimation assay:
All fruit peel extracts were added to 0.5g $BaCl_2$ with 5ml of distilled water and kept for incubation and the absorbance was read at610 nm and the %concentration was estimated.

Pectin assay

Estimation was done calorimetrically in which Galacturonic acid and Carbazole in presence of H_2SO_4 , colour developed was measured at 520 nm. The crude pectin of 100 mg dissolved in 100 mL of 0.05 N of NaOH and incubated for 30 min to de-esterify the pectin. Now the Carbazole reagent (0.1%) of 1ml following 12 mL H_2SO_4 was added to the de-esterified pectin and was incubated for 10 to 20 min to develop the colour and was measured at 520 nm in spectrophotometer. The % of pectin was estimated from the standard graph.

The statistical analysis of these parameters estimated in the fruit peels was done using a stastical software called Statistics Calculator[10].

RESULTS AND DISCUSSION

This study was mainly carried out to identify the important components that could possibly assign some new uses to the fruit peel management rather than them being disposed of as solid wastes or as feed for livestock.

In the experiments accomplished, the highest Protease activity was found in peels of Jujube (2.61%) followed by Pineapple and Papaya Peels (1.84% and 1.58% respectively). Highest concentration of protein was also found in the peels of Kiwi, Avocado and Papaya fruit peels (1.79%,1.57% and1.55%respectively) where as the highest antioxidant concentration was observed in the peels of the of Gooseberry and Guava (2.39% and 1.89%). Also the maximum levels of melanin formation was noted in the peels of Mango, Apple , Papaya and Water melon (2.19% ,2.21%,1.63%, and1.92%).Highest Glucosinolate percentage concentration was found in Guava, Orange, Pomegranate, and Pineapple peels (3.87%,2.91%,2.97% and 2.84% respectively). Elevated levels of % concentration pectin were also noted in Papaya, Muskmelon and Pineapple peels(5.32%, 5.02%, and 4.95% respectively).

Where as the lowest levels of Protease were found in Pomegranate and Watermelon peels (0.30% and 0.21%). Interestingly also lower Protein levels were also found noted in Pomegranate and watermelon (0.29% and 0.34%).Antioxidants concentrations was also lower in Watermelon and Avocado peels (0.08% each). Melanin concentration was low in Guava , Avocado (0.19% each). Glucosinlate levels were found to be low in Apple and Avocado peels (0.81% and 0.89%).Pectin % concentration was low in Apple and Sapota (0.83% and 1.53%)

Figure 1: Graph showing various parameters estimated in fruit peels

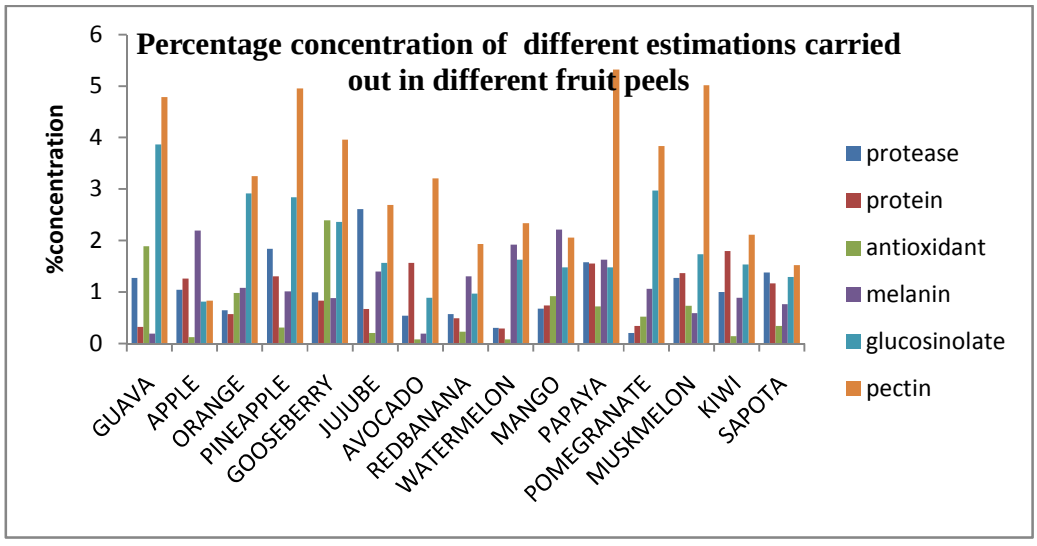


Table 1.Estimation of Protease, Protein,Antioxidant, Melanin, Glucosinolate and Pectin in different fruit peels

%Concentration / Fruit peels	Protease	Protein	Antioxidant	Melanin	Glucosinolate	Pectin
Guava	1.27	0.32	1.89	0.19	3.87	4.79
Apple	1.04	1.26	0.12	2.19	0.81	0.83
Orange	0.64	0.57	0.98	1.08	2.91	3.25
Pineapple	1.84	1.32	0.31	1.01	2.84	4.95
Gooseberry	0.99	0.83	2.39	0.88	2.36	3.96
Jujube	2.61	0.67	0.22	1.42	1.56	2.69
Avocado	0.54	1.57	0.08	0.19	0.89	3.21
Redbanana	0.57	0.49	0.23	1.33	0.97	1.93
Water Melon	0.30	0.29	0.08	1.92	1.63	2.34
Mango	0.68	0.74	0.92	2.21	1.48	2.06
Papaya	1.58	1.55	0.72	1.63	1.43	5.32
Pomegranate	0.21	0.34	0.52	1.06	2.97	3.83
Muskmelon	1.27	1.37	0.73	0.59	1.73	5.02
Kiwi	1.00	1.79	0.14	0.89	1.53	2.11
SAPOTA	1.38	1.17	0.34	0.76	1.29	1.53

Figure 2: Graph showing concentration of protease in various fruits

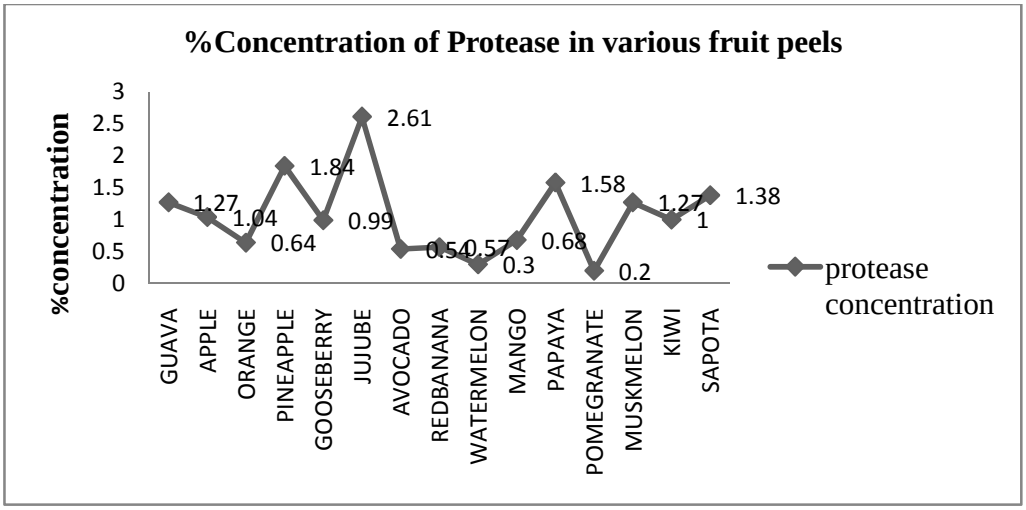


Figure 3: Graph showing %concentration of Protein in various fruit peels

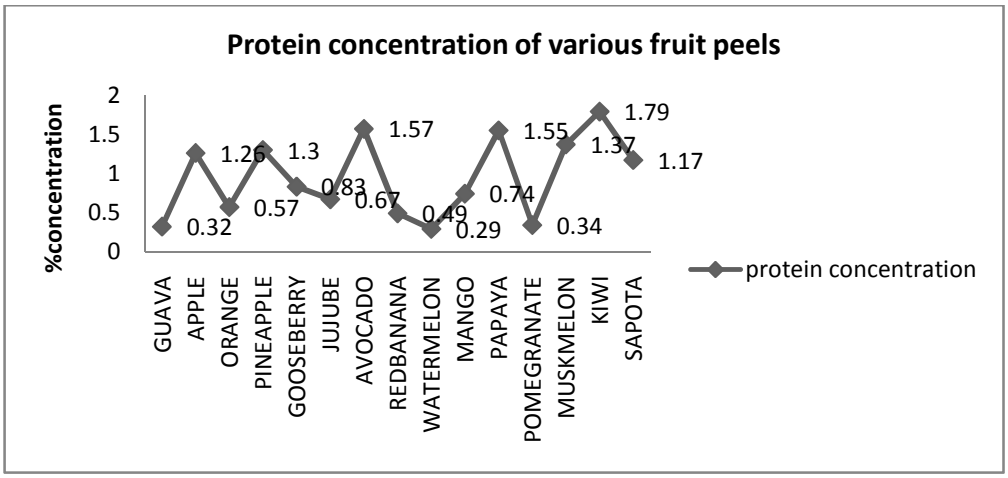


Figure 4: Graph showing %concentration of Antioxidants in various fruit peels

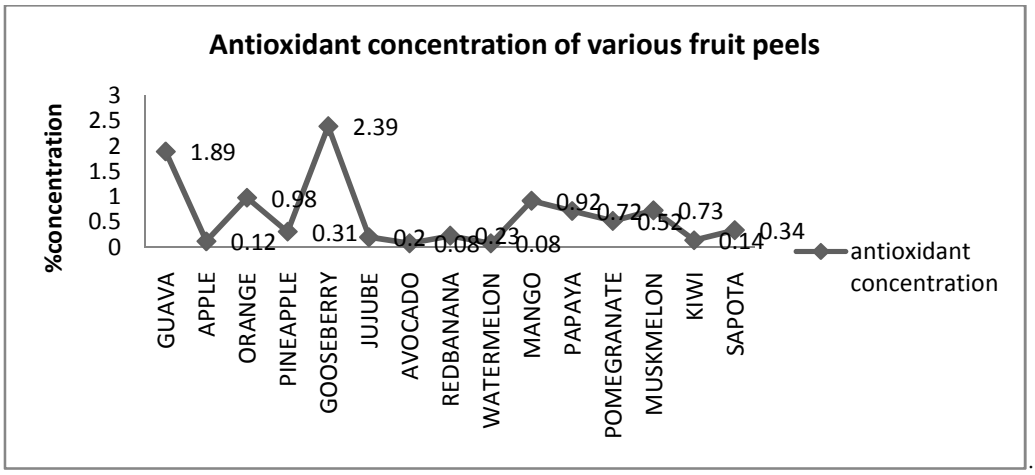


Figure 5: Graph showing %concentration of Melanin in various fruit peels

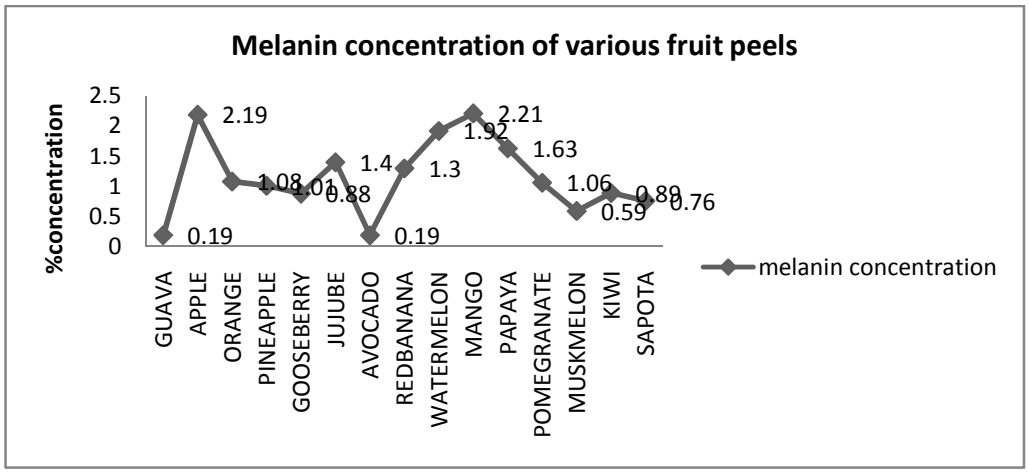


Figure 6: Graph showing %concentration of Glucosinolate in various fruit peels

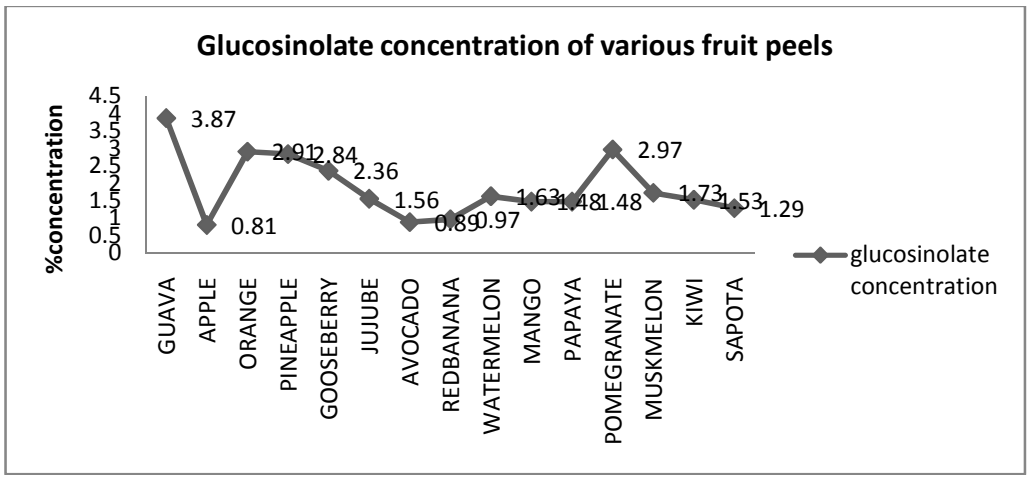


Figure 7: Graph showing %concentration of Pectin in various fruit peels

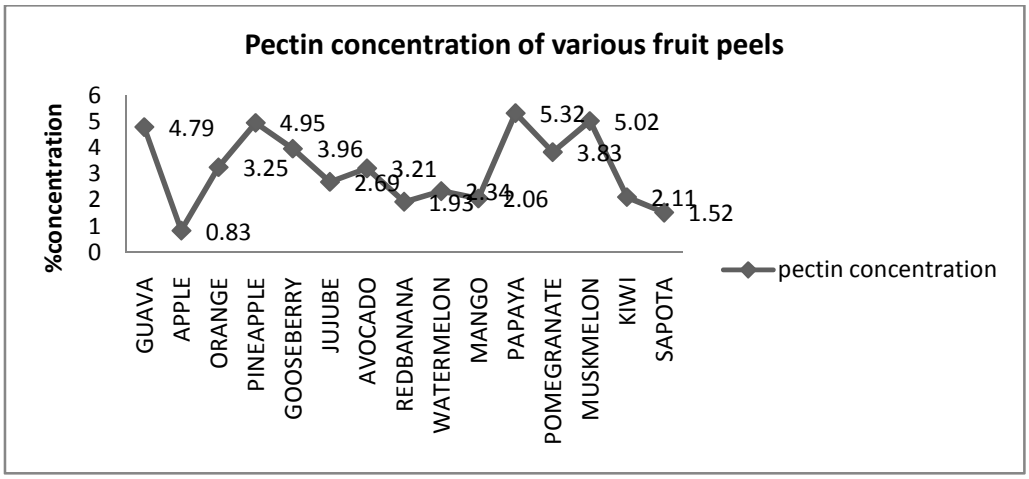


Figure 8: Collection of samples

Figure 9: Estimation of Protease in fruit peels

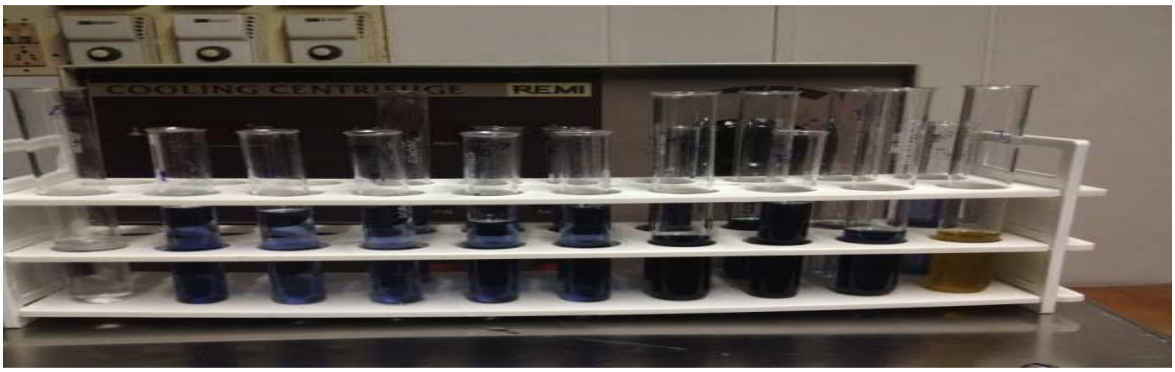


Figure 10: Estimation of Protein by lowry's method in fruit peels

Figure 11:Estimation of Antioxidants in fruit peels

Figure 12:Estimation of Melanin in fruit peels

Figure 13:Estimation of Glucosinolate in fruit peels

Chi Square Test of Independence

The null and alternative hypotheses include:

H_0: The six categorical variables (% Protein, %protease, %Antioxidant, %Melanin ,%Glucosinolate and %pectin) are independent.

H_a: The six categorical variables are related and that percentage concentration of one variable will affect the percentage concentration of the other.

Figure 14: Statisical Calculator showing results for the Chi-Square test being performed various fruit peels numbered from 1-15 as of the same order as mentioned in Table 1 for different parameters being estimated from Column A to F.

Figure 14a:

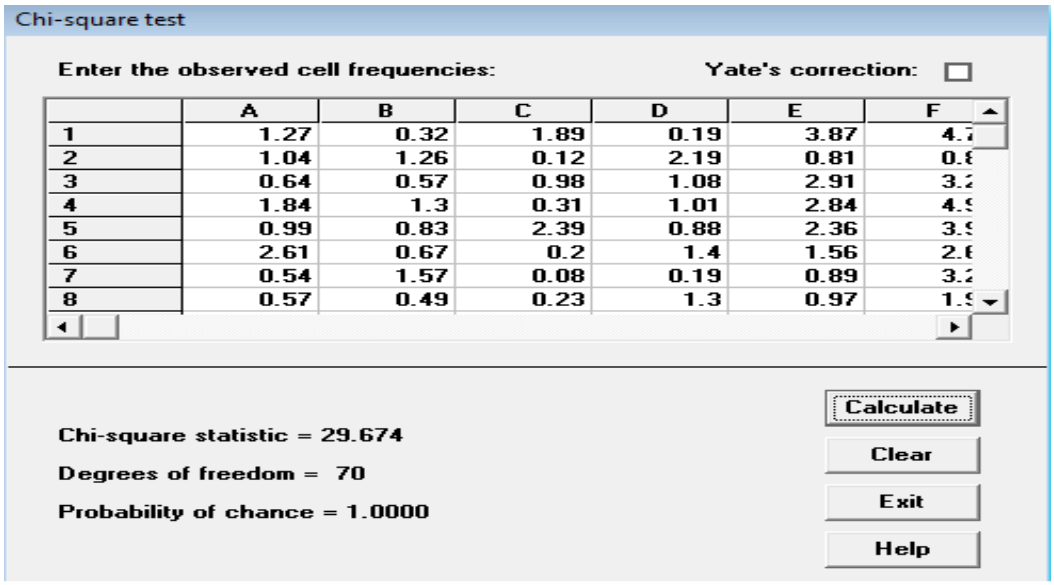


Figure 14b:

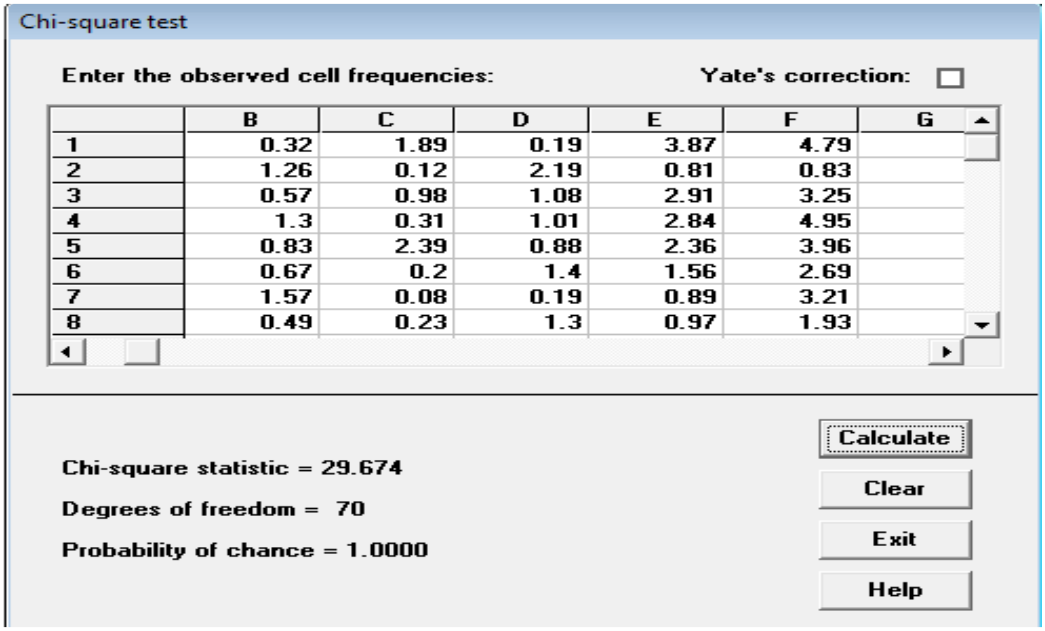


Figure 14c:

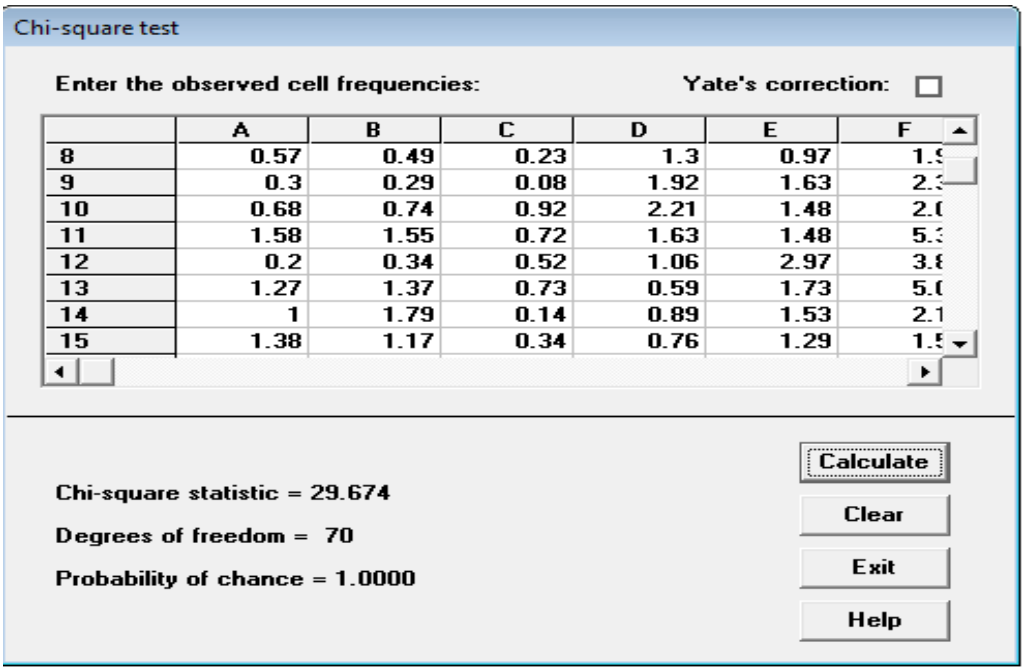


Figure 14d:

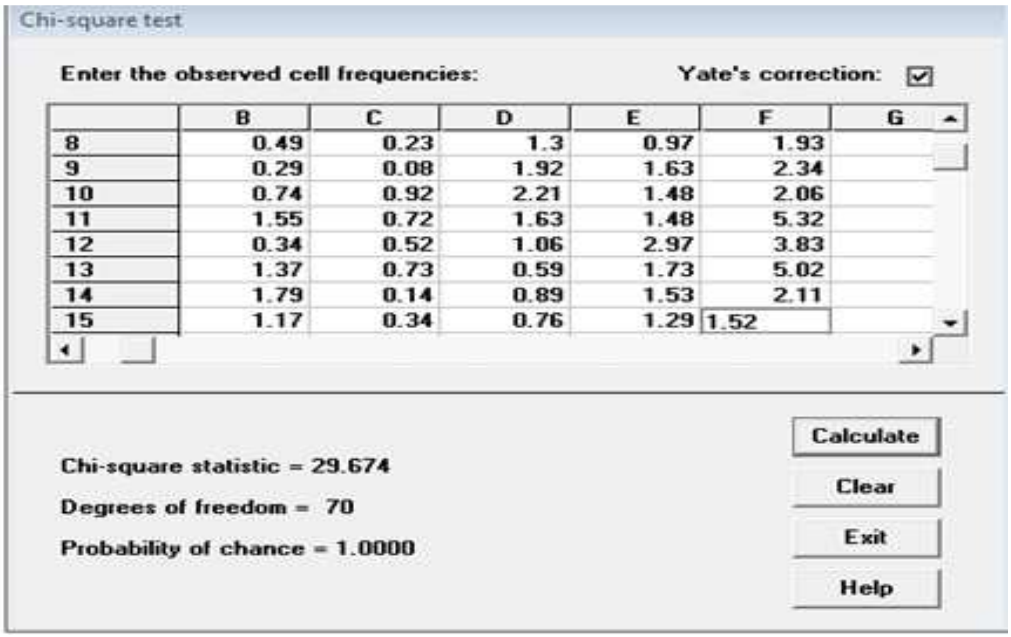
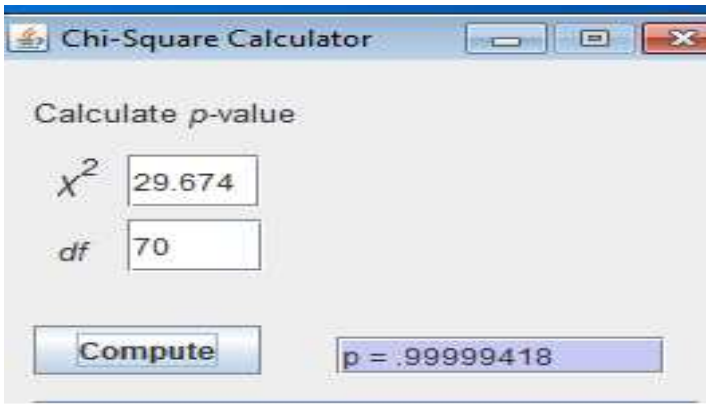


Figure 14e:



As we tried to obtain the difference between the groups of data by chi-square test of independence using statistical softwares called Statistical Calculator and Chi-Square calculator we failed to reject the null hypothesis H_0 with P value <0.99 for the Chi-square test of independence for different estimations made in the fruit peels therefore each of these data are highly independent and presence of one compound or component does not necessarily effect the percentage concentration of the other.

CONCLUSION

The work carried out not only might help us in providing insights into better usage of fruit wastes and help reduction of solid wastes but also helps us in understanding the nature of compounds and their levels in the fruit wastes which might be a important source of information for various pharma, fruit product manufacturing industries.

Acknowledgement
The authors wants to express their gratitude to the management and Dr.G.Viswanathan, hon'ble Chancellor, VIT University, Vellore for constant support and encouragement for providing the infrastructure and good laboratory facilities to carry out this research work and Mr Raj Vuppu , Temple University , Singapore and DST India for financial assistance and support.

REFERENCES

[1] Senthi Kumar and B. N. S. Murthy , *Agrobios News Letter*, **2004**, 2, 9 , 50-52
[2] E. Tripoli,, M. La Guardia, S. Giammanco, D. Di Majoand ,M. Giammanco, *Food Chemistry*,**2007**, 104, 466-479.
[3] S.Sanjay , K.Amod ,V. Suneetha, M.Bishwambhar., R.Gopinath , Y.Sharad and M.Bhaskar *International Journal of Drug Development & Research*, **2012**,4,304-310..
[4] K. Sudhakar , C. Vijaya laxmi , N.L.Gowri Shankara, L.Matsyagiria ,M. Shankar and B.S. Sandhya, Pelagia Research Library, *Der Pharmacia Sinica*, **2011**,3,193-199
[5] H.A. Heikal , M.H. El-Sidawi , A.El-Wakeil , *Agric Research and Reviews*, **1972**,50, 199-214.
[6] W. Kazumasa and I.Shosuke ,*Pigment Cell Research*, **2002**,15, 174-183
[7] R.J. Mailer , *Australian Journal of Agricultural Research*,**1989**, 40, 617–624
[8] V. Suneetha, M.Bishwambhar,R.Gopinath ,S.R.Shrestha ,G.K.B. Kartik , C. Pravesh ,C. Apoorvi,R. Kalyani ,*Asian Journal of Microbiology Biotechnology and Environmental Sciences*,**2012**, 14,405-412.
[9] V.Suneetha, Sindhuja, and K.Sanjeev, *Asian Journal of Microbiology Biotechnology and Environmental Sciences*, **2010**, 12 ,149-155.
[10] V.Suneetha and V.Raj , *International Journal of Drug Development & Research*,**2012**, 4,1-6.