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Research Article

Effective Extraction of Polyphenols from Onion and Pomegranate Peels and Estimation of Radical Scavenging Activity of Extracted Polyphenols

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Abstract

Vegetable peels are usually thrown out as waste, when they usually contain significant amounts of polyphenols. The main aim of this study was to establish an effective extraction method of polyphenols and study their radical scavenging activity. From the results we can clearly infer that 70°C, 40 mins and Ethanol solvent are the best extraction parameters, when considered individually (not in combination), also we can see that for extraction of total phenolics from pomegranate peels ethanol could be the best solvent. In case of onion peels, the maximum yield was obtained from extraction with distilled water. From the observations, we have also calculated the averages of the total phenolic content at different storage temperatures. At -25, the degradation of phenolics was least and when stored in room temperature the degradation of total phenolic content was the most. Thus, we performed DPPH Assay to determine the radical scavenging activity of the phenolic extracts which were stored at -25°C (as degradation of phenolics were least in this storage temperature). Phenolics extracted from onion peels at 50°C for both 20 mins and 40 mins extracted in distilled water showed a radical scavenging activity of 92%. Phenolics extracted from pomegranate peels at 60°C for 60 min extracted in distilled water showed a radical scavenging activity of 91.2%.

Keywords: *Polyphenols; Onion; Pomegranate; Scavenging Activity*

Introduction

Natural antioxidants are preferred over synthetic antioxidants because of presumed safety, potential nutritional benefits and therapeutic effects [1]. Among all the natural antioxidants, polyphenols gain significance owing to their high redox potential which allows them to act as reducing agents, hydrogen donors and singlet oxygen quenchers [2]. Moreover, polyphenols are abundantly present in our diet [3]. The benefits of dietary polyphenols have been studied extensively over the last decade. They have been established to play a significant role in the prevention of degenerative diseases like cancer, cardiovascular, neuro diseases, inflammation, high blood pressure, cholesterol increase [4] etc. Fruits, vegetables, leguminous plants and some cereals are rich sources of polyphenols. Polyphenols are often present in higher concentration in the outer non edible part of the fruits like peel compared to inner edible part [5].

Different drying techniques, extraction temperatures, solvents and duration were attempted. All the extracts were analyzed for total polyphenol content by Folin-Ciocalteu method.

Here we took one fruit and one vegetable peel for our study. For vegetable peel we took peels of onion [6-9].

Materials and Methods

Materials and reagents required

- For total phenolic content, we required:
    - 7.5% Sodium carbonate
    - 1:10 Folin-Ciocalteu Reagent
    - Distilled water.
- For DPPH, we required:
    - DPPH (2,2-diphenyl-1-picrylhydrazil)
    - 0.1M Tris HCL
    - Chilled ethanol
    - Distilled water.
- General requirements:
    - Microcentrifuge tubes
    - Micropipettes
    - Micropipette tips
    - Falcon tube
    - Water bath
    - Beaker
    - Measuring Cylinder
    - Spectrophotometer
    - 96 well Elisa plate
    - Aluminium foil
    - Weighing balance.

Methodology

Sample preparation

- Fresh pomegranates and onions were cleaned with water and dried with a cloth.

- The peels were manually separated, dried for a few days in an open air shade.
- The dried samples were then powdered in a blender.
- They were stored at -18°C until analysis.

Extraction procedure

- Pomegranate and onion peels were placed in a thermostatic water bath shaker with 10 ml of DI water at 50°C for 20 min.
- The liquid extract was separated from solids by centrifugation at 2000 rpm for 10 min.
- The supernatant was transferred to a 10 ml flask.
- DI water was added to make the final volume 10 ml.
- The total polyphenols concentrations as well as the radical scavenging activity were measured.

DPPH assay

This is an assay for scavenging free radical. The scavenging activity of natural products can be assayed by measuring the decrease in absorbance at 517nm of the stable free radical with scavenger to yield the colourless product 1,1 diphenyl-2-picrylhydrazine. The procedure is as follows:

- 500 µM DPPH solution was made in chilled ethanol.
- A stock of 10 mM Ascorbic acid was made.
- 0.1M Tris HCL buffer (pH 7.4) was prepared.
- To 2 ml MCT tubes 100 µl of sample was added.
- To that 500 µl of DPPH was added followed by 400 µl Tris HCL buffer.
- 1 tube was taken as control with water.
- From the ascorbic acid stock, 3 standards were prepared (1 -undiluted, 1 - 10 times diluted and 1 - 100 times diluted).
- All the tubes were in dark for 20 minutes.
- 200 µl from each MCT was loaded on a 96 well ELISA plate.
- Spectrophotometer readings were taken (517 nm)

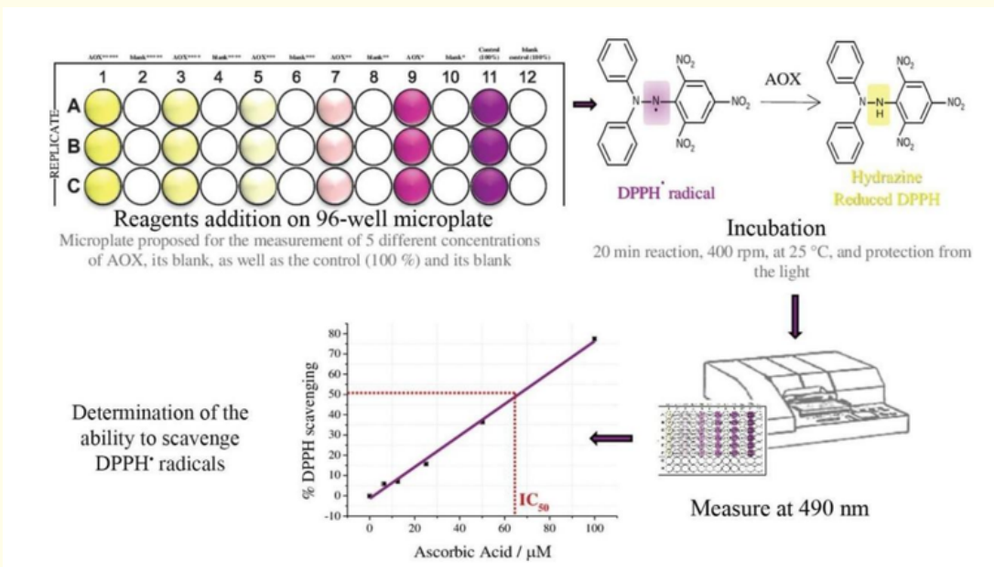

Figure 1

Total phenolic content

The total phenolic content quantification assay is based on Folin-Ciocalteu method. The FC Reagent contains phosphomolybdic/phosphotungstic acid complexes. The method relies on the transfer of electrons in alkaline medium from phenolic compounds to form a blue chromophore constituted by a phosphotungstic/phosphomolybdenum complex where the maximum absorption depends on the concentration of phenolic compounds. The procedure is as follows:

- Make a stock solution of Gallic acid of 500 µg/ml concentration.
- Standards of 50, 100, 150, 200, 250 µg/ml concentration are prepared.
- To 100 µl of sample, 500 µl of FC reagent (Reagent A) should be added and incubated for 5 to 10 minutes in the dark.
- Then, 400 µl of 7.5% sodium carbonate was added and incubated for 1 hour.
- Then spectrophotometric readings are taken at 765 nm.
- Total phenolic content is measured as limits expressed as GAE µg/ml (Gallic acid equivalent).

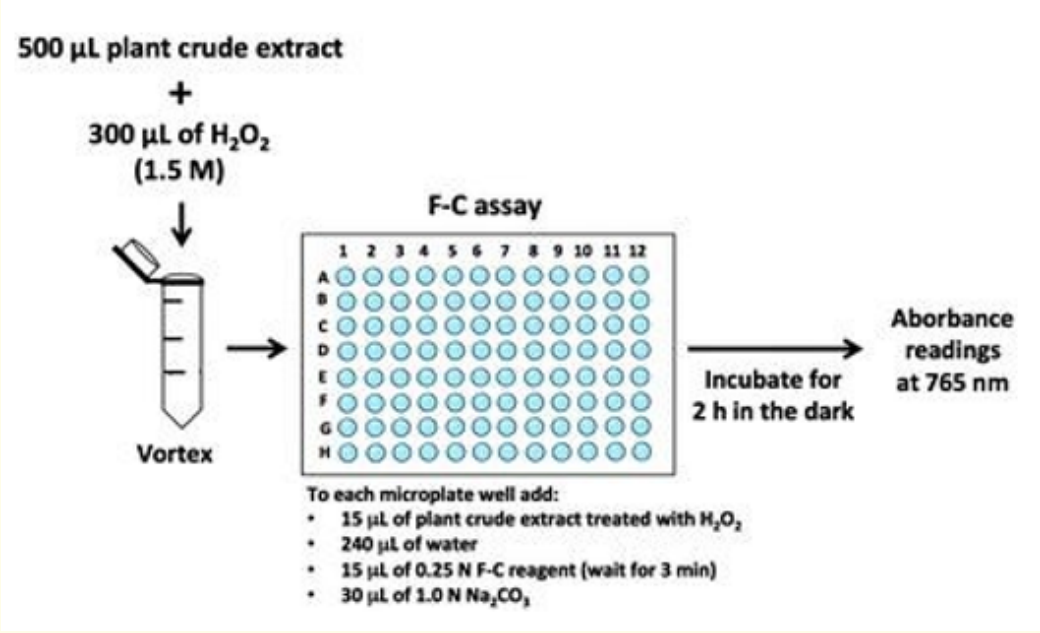

Figure 2

Observations

SL. No.	Concentration (ug/ml)	Absorbance (765 nm)
1	0	0
2	50	0.184
3	100	0.252
4	150	0.497
5	200	0.767
6	250	0.943
7	500	1.865

Table: *Standard curve for total phenolic content.*

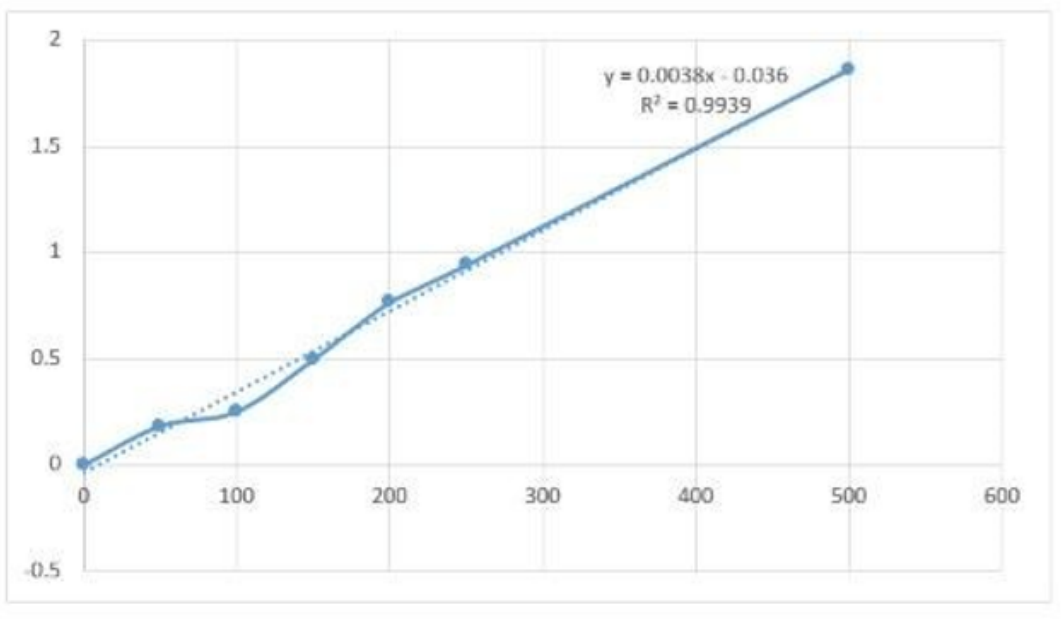

Figure 3: *Graph showing standard curve for total phenolic content.*

Total phenolic content assay

Extraction material	Temperature (RT)	Time (in mins)	Solvent	Total phenol content (ug/ml)
Onion peels	50	20	Distilled water	296.54
Onion peels	50	40	Distilled water	275.62
Onion peels	50	60	Distilled water	Invalid data
Onion peels	50	20	Ethanol	359.18
Onion peels	50	40	Ethanol	Invalid data
Onion peels	50	60	Ethanol	389.17

Onion peels	60	20	Distilled water	303.2
Onion peels	60	40	Distilled water	465.0
Onion peels	60	60	Distilled water	Invalid data
Onion peels	60	20	Ethanol	410.85
Onion peels	60	40	Ethanol	415.03
Onion peels	60	60	Ethanol	416.045
Onion peels	70	20	Distilled water	240.25
Onion peels	70	40	Distilled water	341.52
Onion peels	70	60	Distilled water	355.74
Onion peels	70	20	Ethanol	540.83
Onion peels	70	40	Ethanol	401.20
Onion peels	70	60	Ethanol	420.8

Table 1: *Onion peels at room temperature (Total phenolic content).*
Spectrophotometer readings were taken at (517 nm).
For each sample, 3 dilutions were considered: undiluted, 10^{-1}, 10^{-2}, 10^{-3}.

Extraction material	Temperature (degree C)	Time (in mins)	Solvent	Total phenol content (ug/ml)
Pomegranate	50	20	Distilled water	757.98
Pomegranate	50	40	Distilled water	742.58
Pomegranate	50	60	Distilled water	735.27
Pomegranate	50	20	Ethanol	640.53
Pomegranate	50	40	Ethanol	678.37
Pomegranate	50	60	Ethanol	575.74
Pomegranate	60	20	Distilled water	752
Pomegranate	60	40	Distilled water	704.21
Pomegranate	60	60	Distilled water	698.47
Pomegranate	60	20	Ethanol	773.37
Pomegranate	60	40	Ethanol	756
Pomegranate	60	60	Ethanol	728
Pomegranate	70	20	Distilled water	798.69
Pomegranate	70	40	Distilled water	728.22
Pomegranate	70	60	Distilled water	788.25
Pomegranate	70	20	Ethanol	798.43
Pomegranate	70	40	Ethanol	753.28
Pomegranate	70	60	Ethanol	822.70

Table 2: *Pomegranate peels at room temperature (Total phenolic content).*
Spectrophotometer readings were taken at (517 nm).
For each sample, 3 dilutions were considered: undiluted, 1^{0-1}, 10^{-2}, 10^{-3}.

Extraction material	Temperature (degree C)	Time (in mins)	Solvent	Total phenol content (ug/ml)
Onion peels	50	20	Distilled water	221.89
Onion peels	50	40	Distilled water	297.324
Onion peels	50	60	Distilled water	600.86
Onion peels	50	20	Ethanol	231.29
Onion peels	50	40	Ethanol	610.64
Onion peels	50	60	Ethanol	571.108
Onion peels	60	20	Distilled water	754.84
Onion peels	60	40	Distilled water	782.77
Onion peels	60	60	Distilled water	762.41
Onion peels	60	20	Ethanol	768.0
Onion peels	60	40	Ethanol	758.24
Onion peels	60	60	Ethanol	779.121
Onion peels	70	20	Distilled water	555.60
Onion peels	70	40	Distilled water	506.120
Onion peels	70	60	Distilled water	511.0
Onion peels	70	20	Ethanol	527.26
Onion peels	70	40	Ethanol	517.08
Onion peels	70	60	Ethanol	534.56

Table 3: *Onion peels at 4 degree C (Total phenolic content).*
Spectrophotometer readings were taken at (517 nm).
For each sample, 3 dilutions were considered: undiluted, 10-1, 10-2, 10-3.

Extraction material	Temperature (degree C)	Time (in mins)	Solvent	Total phenol content (ug/ml)
Pomegranate	50	20	Distilled water	318.9
Pomegranate	50	40	Distilled water	241.21
Pomegranate	50	60	Distilled water	348.74
Pomegranate	50	20	Ethanol	398.06
Pomegranate	50	40	Ethanol	481.325
Pomegranate	50	60	Ethanol	514.73
Pomegranate	60	20	Distilled water	304.63
Pomegranate	60	40	Distilled water	317.94
Pomegranate	60	60	Distilled water	340.64
Pomegranate	60	20	Ethanol	386.84
Pomegranate	60	40	Ethanol	334.5
Pomegranate	60	60	Ethanol	346.1
Pomegranate	70	20	Distilled water	947.201
Pomegranate	70	40	Distilled water	898.91
Pomegranate	70	60	Distilled water	961.03
Pomegranate	70	20	Ethanol	979.2
Pomegranate	70	40	Ethanol	979.82
Pomegranate	70	60	Ethanol	954.77

Table 4: *Pomegranate peels at 4 degree C temperature (Total phenolic content).*
Spectrophotometer readings were taken at (517 nm).
For each sample, 3 dilutions were considered: undiluted, 10^{-1}, 10^{-2}, 10^{-3}.

Extraction material	Temperature (degree C)	Time (in mins)	Solvent	Total phenol content (ug/ml)
Onion peels	50	20	Distilled water	724.31
Onion peels	50	40	Distilled water	800.30
Onion peels	50	60	Distilled water	754.58
Onion peels	50	20	Ethanol	793.21
Onion peels	50	40	Ethanol	741.53
Onion peels	50	60	Ethanol	778.90
Onion peels	60	20	Distilled water	309
Onion peels	60	40	Distilled water	480.28
Onion peels	60	60	Distilled water	508.99
Onion peels	60	20	Ethanol	346.89
Onion peels	60	40	Ethanol	535.35
Onion peels	60	60	Ethanol	332.03
Onion peels	70	20	Distilled water	556.4
Onion peels	70	40	Distilled water	375.62
Onion peels	70	60	Distilled water	398.32
Onion peels	70	20	Ethanol	448.70
Onion peels	70	40	Ethanol	659.06
Onion peels	70	60	Ethanol	759.54

Table 5: *Onion peels at -25 degree C (Total phenolic content).*
Spectrophotometer readings were taken at (517 nm).
For each sample, 3 dilutions were considered: undiluted, 10-1, 10-2, 10-3.

Extraction material	Temperature (degree C)	Time (in mins)	Solvent	Total phenol content (ug/ml)
Pomegranate	50	20	Distilled water	331.74
Pomegranate	50	40	Distilled water	298.7
Pomegranate	50	60	Distilled water	217.19
Pomegranate	50	20	Ethanol	332.6
Pomegranate	50	40	Ethanol	294.45
Pomegranate	50	60	Ethanol	209.36
Pomegranate	60	20	Distilled water	482.80
Pomegranate	60	40	Distilled water	243.03
Pomegranate	60	60	Distilled water	200.14
Pomegranate	60	20	Ethanol	371.1
Pomegranate	60	40	Ethanol	457.2
Pomegranate	60	60	Ethanol	386.06
Pomegranate	70	20	Distilled water	520.99
Pomegranate	70	40	Distilled water	402.24
Pomegranate	70	60	Distilled water	512.90
Pomegranate	70	20	Ethanol	494.6
Pomegranate	70	40	Ethanol	582.33
Pomegranate	70	60	Ethanol	557.53

Table 6: *Pomegranate peels at -25 degree C temperature (Total phenolic content).*
Spectrophotometer readings were taken at (517 nm).
For each sample, 3 dilutions were considered: undiluted, 10-1, 10-2, 10-3.

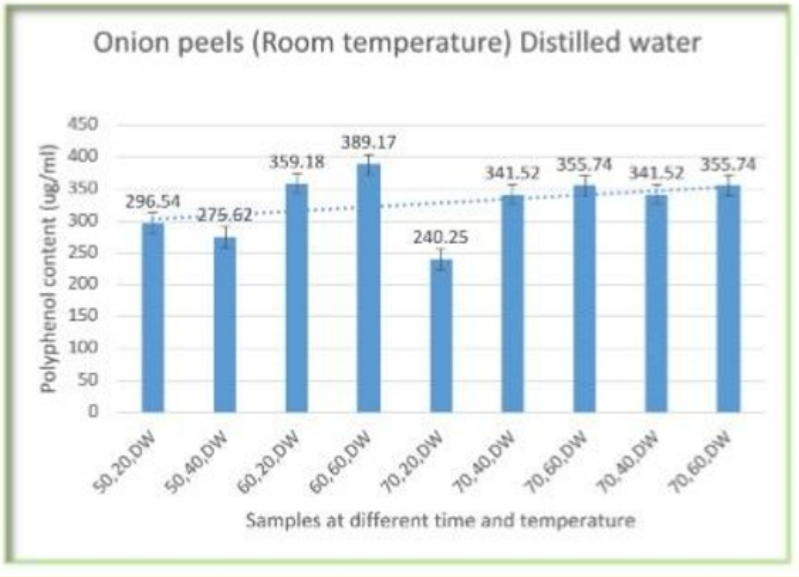

Figure 4: *Estimation of the total polyphenolic content by TPC assay extracted from onion peels in distilled water as the solvent. The extracts were stored at room temperature before performing the assay.*

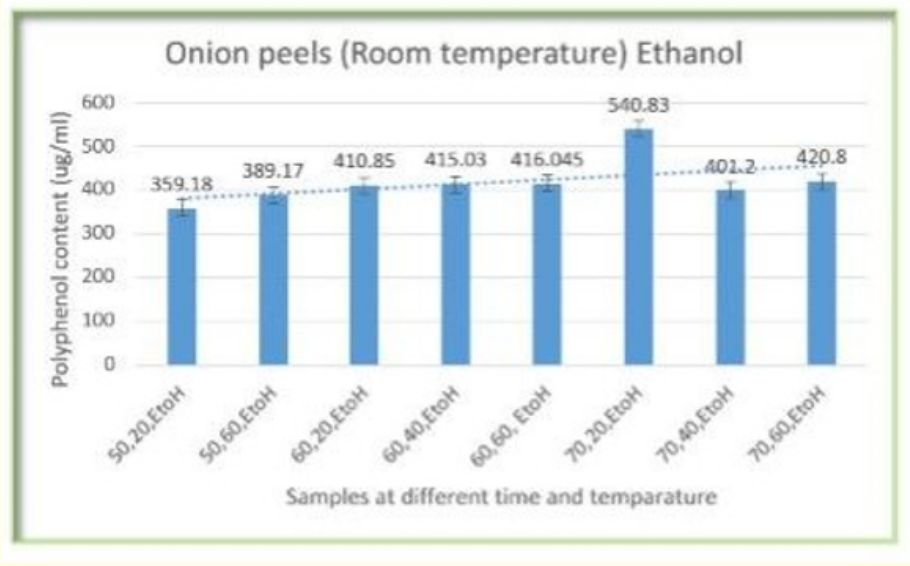

Figure 5: *Estimation of the total polyphenolic content by TPC assay extracted from onion peels in ethanol as the solvent. The extracts were stored at room temperature before performing the assay.*

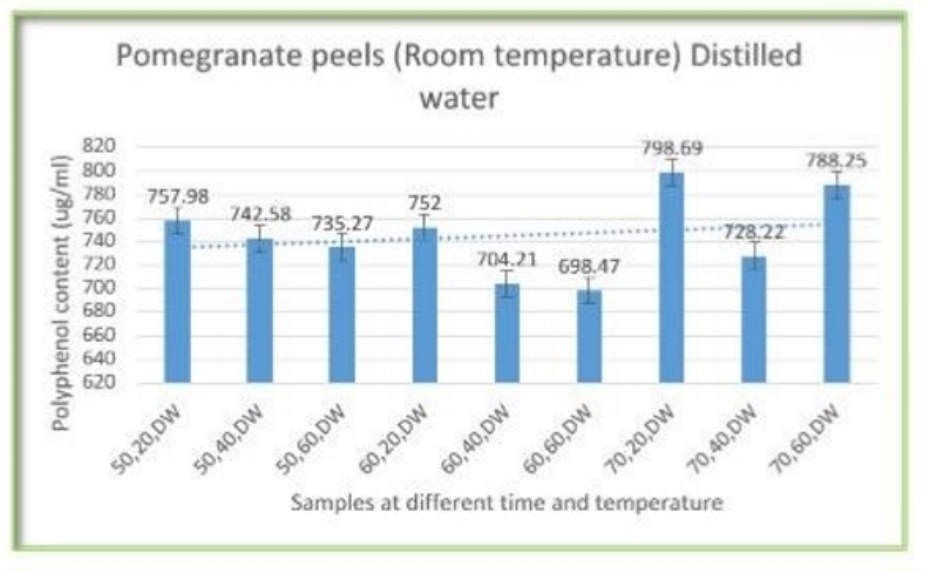

Figure 6: *Estimation of the total polyphenolic content by TPC assay extracted from pomegranate peels in distilled water as the solvent. The extracts were stored at room temperature before performing the assay.*

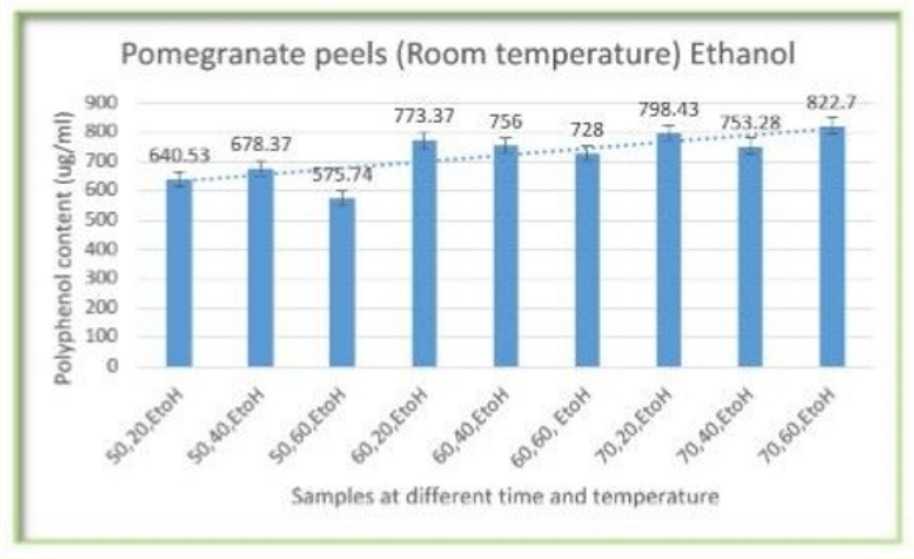

Figure 7: *Estimation of the total polyphenolic content by TPC assay extracted from pomegranate peels in ethanol as the solvent. The extracts were stored at room temperature before performing the assay.*

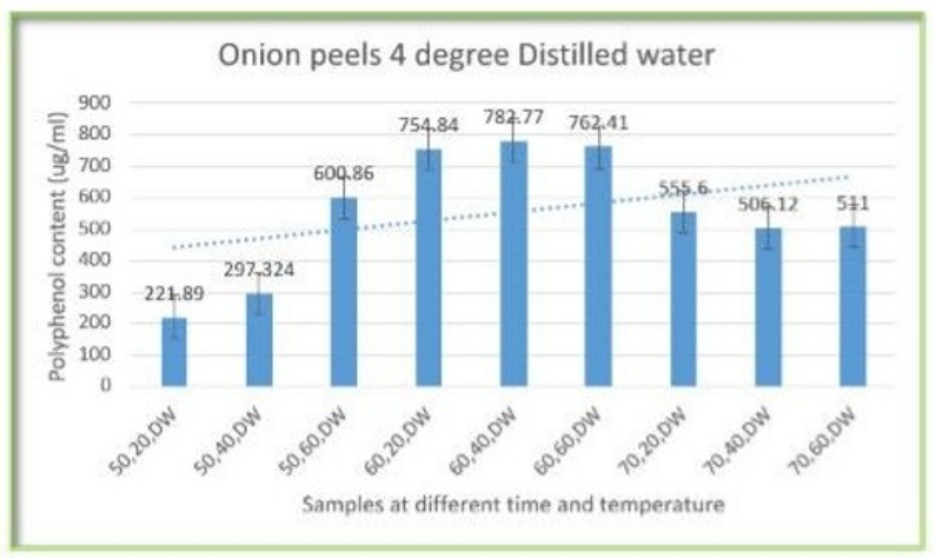

Figure 8: *Estimation of the total polyphenolic content by TPC assay extracted from onion peels in distilled water as the solvent. The extracts were stored at 4 degree C temperature before performing the assay.*

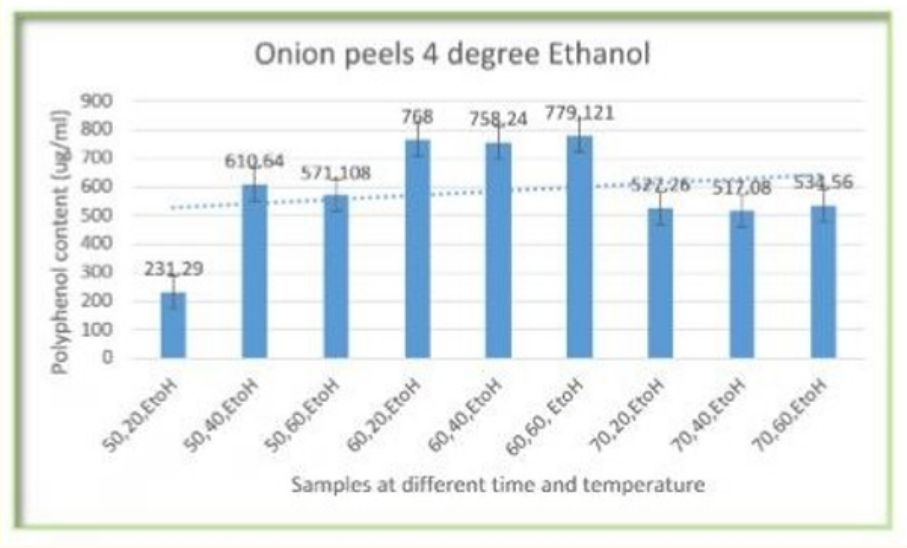

Figure 9: *Estimation of the total polyphenolic content by TPC assay extracted from onion peels in ethanol as the solvent. The extracts were stored at 4 degree C temperature before performing the assay.*

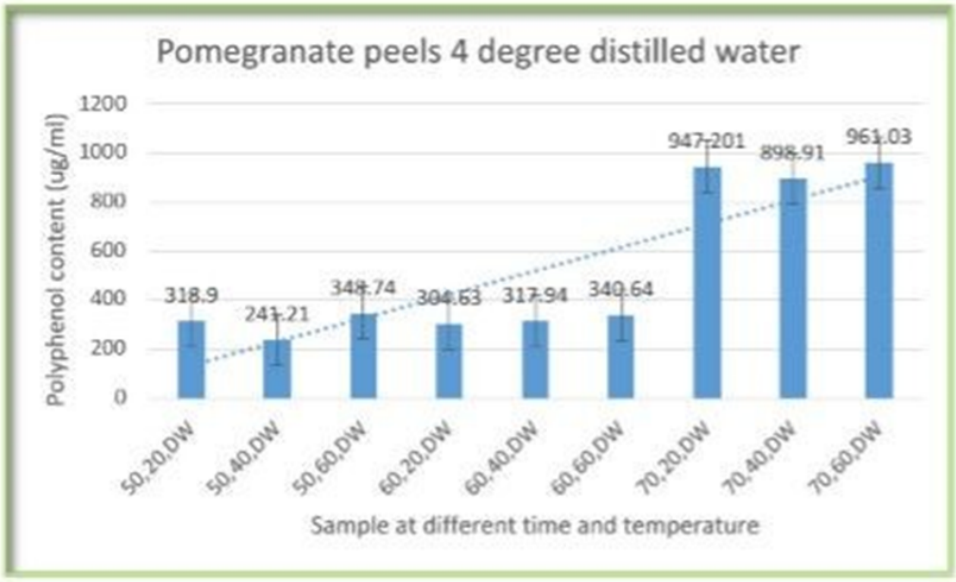

Figure 10: *Estimation of the total polyphenolic content by TPC assay extracted from pomegranate peels in distilled water as the solvent. The extracts were stored at 4 Degree C temperature before performing the assay.*

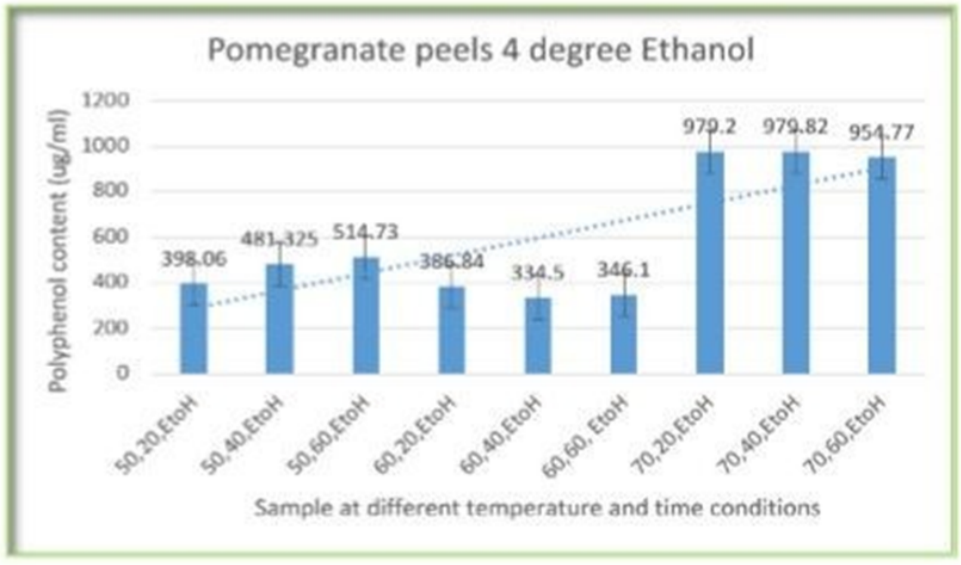

Figure 11: *Estimation of the total polyphenolic content by TPC assay extracted from pomegranate peels in ethanol as the solvent. The extracts were stored at 4 degree C temperature before performing the assay.*

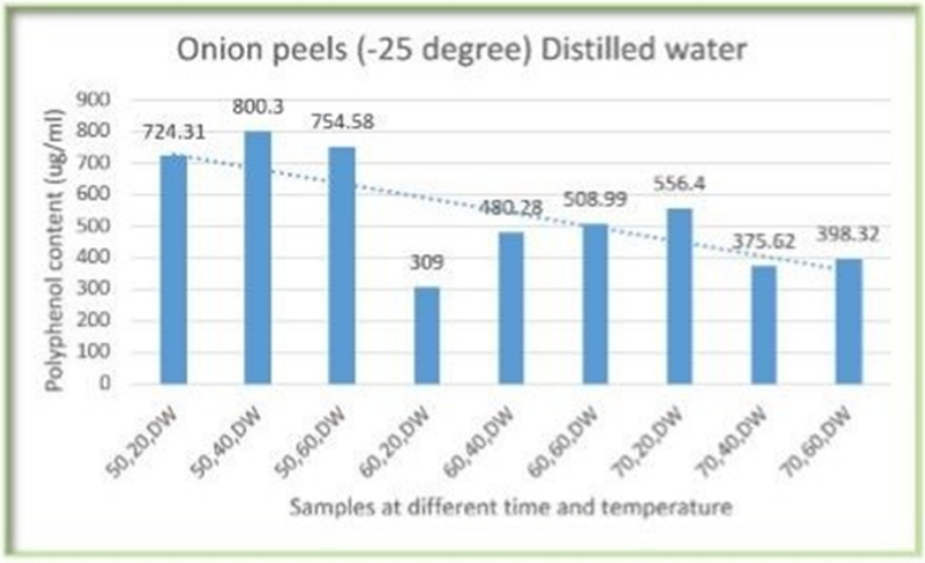

Figure 12: *Estimation of the total polyphenolic content by TPC assay extracted from onion peels in distilled water as the solvent. The extracts were stored at -25 degree C temperature before performing the assay.*

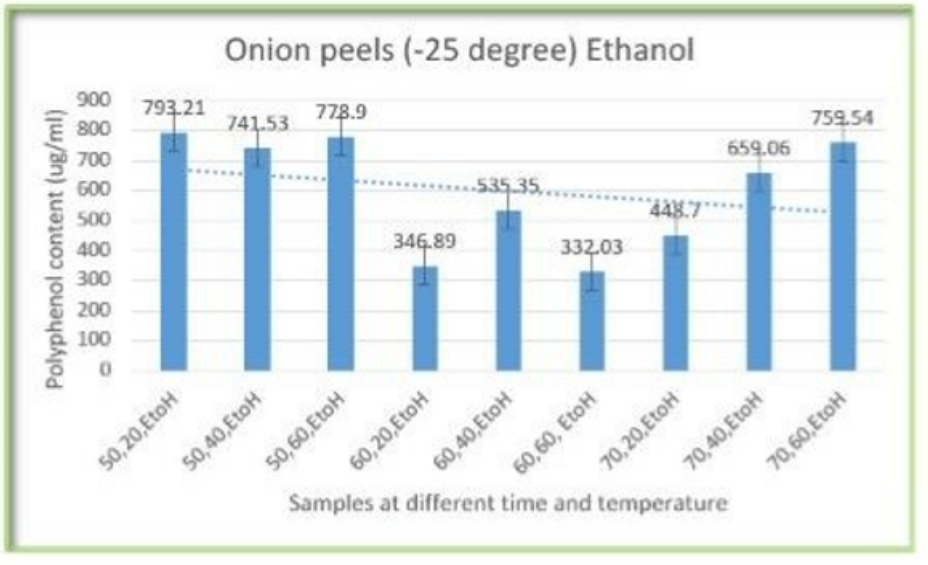

Figure 13: *Estimation of the total polyphenolic content by TPC Assay extracted from onion peels in ethanol as the solvent. The extracts were stored at -25 degree C temperature before performing the assay.*

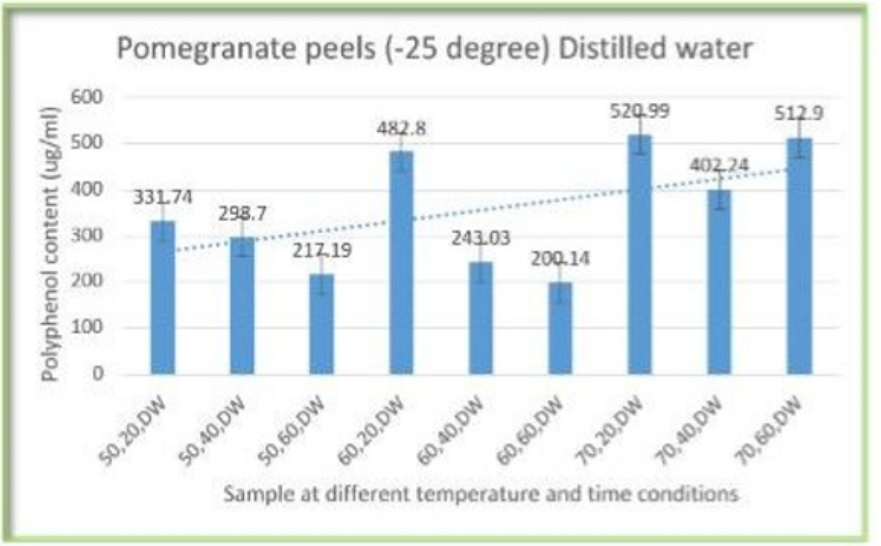

Figure 14: *Estimation of the total polyphenolic content by TPC assay extracted from pomegranate peels in distilled water as the solvent. The extracts were stored at -25 degree C temperature before performing the assay.*

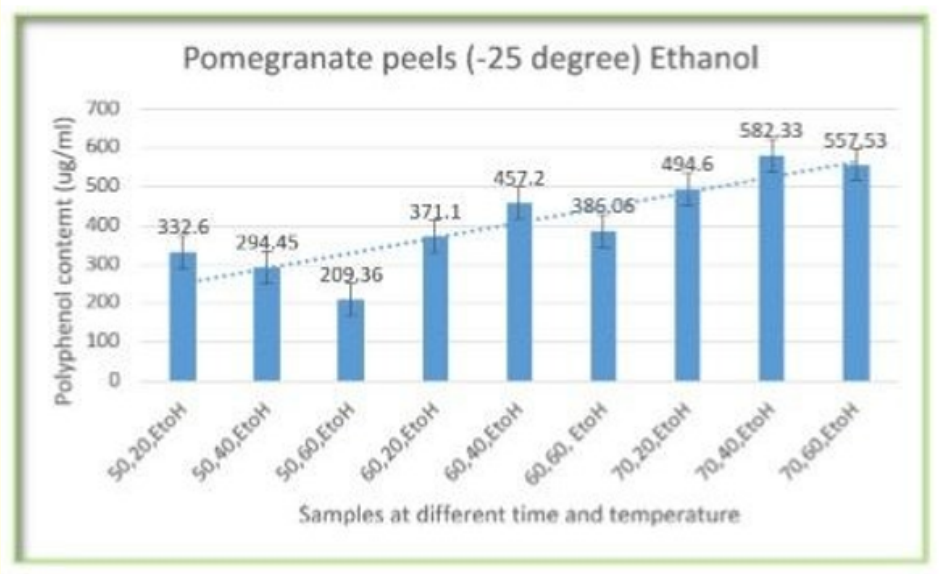

Figure 15: *Estimation of the total polyphenolic content by TPC assay extracted from Pomegranate peels in ethanol as the solvent. The extracts were stored at -25 degree C temperature before performing the assay.*

DPPH assay

Extraction material	Temperature (degree C)	Time (in mins)	Solvent	Scavenging activity (%)
Onion peels	50	20	Distilled water	92
Onion peels	50	40	Distilled water	91
Onion peels	50	60	Distilled water	92
Onion peels	50	20	Ethanol	91.5
Onion peels	50	40	Ethanol	89.9
Onion peels	50	60	Ethanol	90
Onion peels	60	20	Distilled water	90
Onion peels	60	40	Distilled water	87
Onion peels	60	60	Distilled water	89.5
Onion peels	60	20	Ethanol	90.08
Onion peels	60	40	Ethanol	91.2
Onion peels	60	60	Ethanol	89
Onion peels	70	20	Distilled water	88.0
Onion peels	70	40	Distilled water	87.6
Onion peels	70	60	Distilled water	89.45
Onion peels	70	20	Ethanol	88.65
Onion peels	70	40	Ethanol	89.34
Onion peels	70	60	Ethanol	88.90

Table 7: *Sample-onion peels, storage temperature- -25.*

Extraction material	Temperature (degree C)	Time (in mins)	Solvent	Scavenging activity (%)
Pomegranate	50	20	Distilled water	88.92
Pomegranate	50	40	Distilled water	88.0
Pomegranate	50	60	Distilled water	89.76
Pomegranate	50	20	Ethanol	88.5
Pomegranate	50	40	Ethanol	87
Pomegranate	50	60	Ethanol	88.9
Pomegranate	60	20	Distilled water	88.14
Pomegranate	60	40	Distilled water	90.56
Pomegranate	60	60	Distilled water	91.2
Pomegranate	60	20	Ethanol	85.09
Pomegranate	60	40	Ethanol	82.35
Pomegranate	60	60	Ethanol	87.0
Pomegranate	70	20	Distilled water	84.231
Pomegranate	70	40	Distilled water	82.66
Pomegranate	70	60	Distilled water	87.5
Pomegranate	70	20	Ethanol	87.007
Pomegranate	70	40	Ethanol	85.56
Pomegranate	70	60	Ethanol	87.46

Table 8: *Sample-pomegranate peels, storage temperature- -25.*

Results

The Total Phenolic Content from onion peels and pomegranate peels were extracted at different temperatures (50°C, 60°C, 70°C) and times (20 mins, 40 mins, 60 mins) with two different solvents (Ethanol and Distilled water).

From table 1, we can see that the total phenolic content extracted from onion peels is 540.83 µg/ml. This maximum yield was obtained at optimum extraction temperature of 70°C for 20 mins when extracted in ethanol.

From table 2, we can see that the total phenolic content extracted from pomegranate peels is 822.70 µg/ml. This maximum yield was obtained at optimum extraction temperature of 70°C for 60 mins when extracted in ethanol.

Note: The above set of extracts were stored at room temperature for 7 days before performing the TPC Assay as we also checked the effects of storage temperature on Total phenolic content extracted from the sample.

From table 3, we can see that the total phenolic content extracted from onion peels is 782.77 µg/ml. This maximum yield was obtained at optimum extraction temperature of 60°C for 40 mins when extracted in distilled water.

From table 4, we can see that the total phenolic content extracted from pomegranate peels is 979.82 µg/ml. This maximum yield was obtained at optimum extraction temperature of 70°C for 40 mins when extracted in ethanol.

Note: The above set of extracts were stored at 4°C for 7 days before performing the TPC Assay as we also checked the effects of storage temperature on Total phenolic content extracted from the sample.

From table 5, we can see that the total phenolic content extracted from onion peels is 800.30 µg/ml. This maximum yield was obtained at optimum extraction temperature of 50°C for 40 mins when extracted in distilled water.

From table 6, we can see that the total phenolic content extracted from pomegranate peels is 582.33 µg/ml. This maximum yield was obtained at optimum extraction temperature of 70°C for 40 mins when extracted in ethanol.

Note: The above set of extracts were stored at -25°C for 7 days before performing the TPC Assay as we also checked the effects of storage temperature on Total phenolic content extracted from the sample.

From the results we can clearly infer that 70°C, 40 mins and Ethanol solvent are the best extraction parameters, when considered individually (not in combination), also we can see that for extraction of total phenolics from pomegranate peels ethanol could be the best solvent. In case of onion peels, the maximum yield was obtained from extraction with distilled water.

From the observations, we have also calculated the averages of the total phenolic content at different storage temperatures. At -25°C, the degradation of phenolics was least and when stored in room temperature the degradation of total phenolic content was the most.

Thus, we performed DPPH Assay to determine the radical scavenging activity of the phenolic extracts which were stored at -25°C (as degradation of phenolics were least in this storage temperature).

Phenolics extracted from onion peels at 50°C for both 20 mins and 40 mins extracted in distilled water showed a radical scavenging activity of 92%.

Phenolics extracted from pomegranate peels at 60°C for 60 min extracted in distilled water showed a radical scavenging activity of 91.2%.

Discussions

According to conducted research works, the Total phenolic content of onion peel extracts, after extraction with ethanol was found to be 327.5 mg GAE/g extract and of pomegranate peel extracts, after extraction with methanol was found to be 104.6 mg GAE/g extract.

In depth investigations at a molecular level have revealed that quercetin and its glucosides are the most abundant phenolics in onion outer dry layers. The most abundant polyphenols in pomegranate peels are the hydrolyzable tannins called ellagitannins, formed when ellagic acid which binds with a carbohydrate.

We cannot compare our data with the above because our conditions for extraction of polyphenols are different and also we have subjected our extracts to different storage temperatures which resulted in some amount of degradation of phenolics.

Although not exactly, but there is a trend in the results that shows the total phenolic content in onion peels to be more than that in pomegranate peels.

There may be deviations in the results because of experimental errors and handling errors. The storage temperatures are other factors that could result in degradation of some amount of phenolics.

It is quite evident from the results that phenolics when stored in room temperature and in light degrades significantly. Storage at low temperatures like -18°Cor in our case -25°C, is preferable to prevent degradation. We could not maintain the temperature at -18°C because the freezer in our laboratory was set at -25°C and we could not change it due to unavoidable reasons.

We have dried our samples in microwave, keeping in mind the monsoon season which is why we were unable to sun-dry them. Certain amount of phenolics may have got affected due to this reason.

Conclusion

Recent advancements in extraction of phenolics from fruit peels include antioxidants that were extracted from the fruit peels of *A. altilis* that has high antioxidant activity. Phenolics extracted from natural sources like fruit and vegetable peels prove to be better antioxidants than synthetic ones. Phenolics with high antioxidant activity helps in extending the shelf life of food product by preventing the peroxide formation in the product containing fat and oil. Many phenolic extracts like bamboo shoot dietary extracts are used as stabilizers of oils and also acts as anti-browning agents. In addition, natural antioxidants are safe and impart health benefit to the consumer. Phenolic compounds are one of the most important classes of phytochemicals with both functional and health promoting properties. Fruits and vegetable wastes account for about 50 Metric Tones of waste, which could be utilized wisely in extraction of these very important polyphenols which could confer excellent benefits to health and also be advantageous for other industrial usage. Thus, it is a step towards sustainable development as well which could correctly define the phrase of "waste to wealth".

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8. What Are Polyphenols? Types, Benefits, and Food Sources.

9. Polyphenols: Contributors to Good Health – Part 2.

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