PREPARATION AND ANALYSIS OF NUTRITIONAL AND ANTIOXIDANT PROFILE OF LEMON (CITRUS LIMON) PEEL POWDER

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Abstract– Globally, India is the leading producer of fruits. Fruits after consumption leave a peel which is a nuisance to the environment as a solid waste. Hence the study was conducted with the objective, development of lemon peel powder. Lemon peels were collected from juice- center at local market of Vijayapura district, Karnataka. Lemon Peel is derived from the fruit of Citrus limon, the common lemon. Lemon Peel contains calcium, phosphorus, potassium, ascorbic acid and vitamin A, as well as volatile oil and hesperidins. Lemon Peels were sliced, blanched, dried and ground to produce Lemon Peel Powder. This study was done to determine the nutritional and Antioxidant Profile of Lemon Peel Powder. The nutritional composition viz, Ash, Carbohydrates, Crude fibre, Fat, Proteins, and Moisture content of lemon peel powder were analyzed and found 2.20g, 48.14g, 15.11g, 1.52g 7.19g, and 9.75% per 100 g. and the antioxidant profile viz, vitamin c, total polyphenols, total flavonoids, DPPH radical scavenging activity ABTS antioxidant assay and Ferric reducing antioxidant power assay of lemon peel powder were analysed and found 60.37mg, 797.64 mg GAE/100g, 639.57mg CE/100g, 49.56(% inhibition), 38.77 (% inhibition), and 3.19 (µmol Fe(II)/g) respectively.

INTRODUCTION

Vegetables and fruits yield about 25% to 30% of non-edible products such as peels and seeds. In most cases these waste by-products contain high contents of antioxidant and antimicrobial compounds that can be successfully utilized as a source of phytochemicals and antioxidant agents (John et al., 2017) Food processing industry including fruit and vegetable processing is the second largest generat, or of wastes into the environment after the household sewage. A massive amount of waste is produced in the fruit and vegetable processing industries, which contains numerous valuable substances of high nutritive value with large economic potential. The processing of fruits and vegetables results in huge amounts of waste materials such as peels, seeds, stones, and unused flesh generated down the processing chain (Gowe, 2015).

Fruits and vegetables are considered to be basically used food products being either fully cooked, nominally cooked or uncooked. It has been found that the processing of vegetables and fruits alone produces a notable waste of 25–30% of the total product. Furthermore, peels, pomace, rind and seeds are considered to be among the most common wastes (Kumar et al., 2020). Oranges and lemons are important medicinal plants of the family Rutaceae. It is reported that their pulp as well as peel have antibacterial potential in addition to other properties (Parashar et al., 2014). The fruit and vegetable wastes are inexpensive, abundantly available and are a good source of dietary fibre (Alkozai et al., 2018).

MATERIALS AND METHODS

Sample collection: Lemon peels were collected from juice- center at local market of Vijayapura district, Karnataka.

Sample preparation: Lemon peels were washed carefully with tap water to remove dirt soil from surface. The remaining juice was extracted by manually and seeds were removed. After juice
extraction the blanching was done for 3 min at 100 °C. Then peels were, dried under sunlight for 20 hrs, followed by grinding into fine powder and the powder was then sieved using the sieve. Then packaged in polyethylene bags and stored at 4±1°C until required for use.

**Analysis of proximates:** The proximate analysis including moisture, protein, fats, crude fibre, Ash, were carried out by following AOAC official methods of analysis (2005) and The Carbohydrates content was calculated as per Biochemical Methods explained by Manickam and Sadasivam).

**Estimation of Moisture (%):** The stainless steel boxes (weight, W empty) were placed on a top loading balance, and their weights were recorded. The sample was carefully transferred into a stainless steel box using a stainless steel spatula to avoid contamination. The weights of the stainless steel boxes contained the samples on a top loading balance and were recorded as (weight, W initial). The stainless steel boxes were placed into a hot air oven whose temperature was set at 100 °C for 16-18 hr. The weights of the stainless steel boxes contained the samples on a top loading balance and recorded as (weight, W initial). The samples were taken from a hot oven using tongs and placed in a desiccator. The weight of the steel box was recorded after cooling to room temperature and placed in a hot oven for another 4h. Samples were placed into a hot oven using tongs and placed in a desiccator. 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\[
\%N = \frac{14.01 \times (B - T.V.) \times N \times 100}{\text{Sample weight} \times 1000}
\]

Where, N is normality of titrant
B is titer value of blank
T.V. is titer value of sample

Now convert the percent nitrogen into percent protein by multiplying with conversion factor as follows

\[
\% \text{ protein} = \%N \times 6.25
\]

**Estimation of Fat:** Fat is estimated as crude ether extract of the dry material. The dry sample (5-10g) is weighed accurately into a thimble and plugged with cotton. The thimble is then placed in a soxhlet apparatus and extracted with anhydrous ether for about 16 hrs. The ether extract is filtered into a weighed conical flask. The flask containing ether and the washings are also transferred. The ether is then removed by evaporation and the flask with the residue dried in an oven at 80 to 100 °C, cool in a desiccator and weighed.

**Calculation**

Weight of evaporating dish (W1)
Weight of evaporating dish after extraction (W2)
Weight of weight of ether extract (W2-W1)

\[
\text{Fat content g/100g} = \frac{\text{Weight of ether extract}}{\text{Weight of sample}} \times 100
\]

**Estimation of Crude fiber:** Take 1g defatted sample in gouch crucible, Add 200 ml of 1.25% sulphuric acid and boil for 45 minutes at 450 °C, Remove the digested acid solution by draining process, Boil with 200 ml of 1.25% sodium hydroxide for 45 minutes at 450 °C, Remove the digested alkali solution by draining process, Crucibles are dried at 100 °C in hot air oven for 2-3 hours and weighed (W1) Place the crucible in muffle furnace for 2 to 3 hours at 600 °C, cool and weigh (W2), The difference in weight (W1-W2) represents the weight of crude fibre. The percent crude fibre is calculated by formula

\[
\% \text{ crude fibre} = \frac{(W1-W2) \times 100}{\text{Weight of sample}}
\]

**Estimation of Ash Content:** About 5 to 10 g of the
sample was weighted accurately into a crucible (which was previously heated to about 600 °C and cooled). The crucible was placed on a clay pipe triangle and heated first over a low flame till all the material was completely charred, followed by heating in a muffle furnace for about 3-5 hours at about 600 °C. It was then cooled in a desiccator and weighted. To ensure completion of ashing the crucible was again heated in the muffle furnace for ½ hour, cooled and weighed. This was till consecutive weights were obtained and the ash was almost white or grayish white in color.

\[
\text{Ash content of sample /100g} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100
\]

**Estimation of Total Carbohydrates:** Weigh 100 mg of the sample into a boiling tube. Hydrolyze by keeping it in a boiling water bath for three hours with 5 ml of 2.5 N HCl and cool to room temperature. Neutralize it with solid sodium carbonate until the effervescence ceases. Make up the volume to 100 ml and centrifuge. Pipette out 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the working standard into a series of test tubes. Pipette out 0.1 and 0.2 ml of the sample solution in two separate test tubes. Make up the volume in each tube to 1 ml with water. Set a blank with 1 mL with water. Add 1 ml of phenol solution to each tube. Add 5 ml of 96% sulphuric acid to each tube and shake well. After 10 min shake the contents in the tubes and place in a water bath at 25-30 ºC for 20 min. Read the color at 490 nm. Calculate the amount of total carbohydrate present in the sample solution using the standard graph.

**Calculation**
The amount of total carbohydrate in the sample was calculated using the standard graph.

Absorbance corresponds to 0.1 ml of the test = \( \frac{x}{0.1} \) mg of glucose

\[
\text{Total carbohydrate (g/100g)} = \frac{x}{0.1} \times 100
\]

= % of total carbohydrate present

**Analysis of Minerals by Atomic Absorption Spectrophotometer (AAS)**

**Estimation of Fe, Ca, Zn, Na and K:** These minerals were estimated using AOAC official methods of analysis 2005.

**Procedure:** Weigh 1.0g of homogenous sample in duplicates into PTFE digestion tubes. Add 3 ml of Suprapur HNO\(_3\) (65%), mix on a vortex and leave it for predigestion for 10 minutes. Add 1ml of Suprapur H\(_2\)O\(_2\) and mix and leave for predigestion for 20 minutes. Close the PTFE tubes and put it for digestion in CEM – MARS - digestion system. After digestion, the clear solution is transferred to 25ml standard flasks and the final volume is made up to 25ml with Milli Q H\(_2\)O after thorough rinsing of the tube. The solution is filtered through Whatman no.42 filter paper and the solution is used for analysis in AAS. A set of each element standards in the range (0.1µg/ml to 5 µg/ml) depending on the range of concentration from a stock of 1000 µg/ml.

**Calculation**

Mineral element (mg/100g) = Vol.made up in ml x Conc. in µg/ml x Dilution \( \times 100 \)

Wt of sample in gms \( \times 1000 \)

Where, 100 is for 100g of food sample, 1000 is for converting µg to mg

**Estimation of Phosphorous:** Phosphorous was calculated using AOAC official method, 2000. Weigh 0.5 to 1.0g of homogenous powdered sample/ CRM / RM in duplicates into PTFE digestion cells. Add 3 ml of Suprapur HNO\(_3\) (65%), mix on a vortex and leave it for predigestion for 10 minutes. Add 1ml of Suprapur H\(_2\)O\(_2\), mix and close the PTFE tubes and Leave for predigestion for 20 minutes in the protective turn table. Put it for digestion in CEM – MARS - digestion system. The above procedure is also done for blank with no sample added to it. Microwave digest the samples similar to other elements. After digestion, the clear solution is transferred to 25ml standard flasks and the final volume is made up to 25 ml with Milli Q H\(_2\)O after thorough rinsing of the digestion Tubes. The solution is filtered if required using Whatman No.42 filter paper. The digested sample / CRM/ RM/ Blank is diluted with MilliQ water & then taken for analysis of Phosphorus by modified Fiske and Subbarow method. A set of standards in the range of (20µg to 120µg) is prepared from a stock of 100µg/ml. Take required no. of test tubes and label them as blank, standards, samples etc. Add the respective solution (standard or samples) in the required concentration as given in the protocol. Then add MilliQ water to make up the volume to 12 ml. Add 1 ml of ammonium molybdate, 1ml of hydroquinone and 1ml of Na\(_2\)SO\(_3\) in each tube, mix well and incubate at room temperature for 30min. Take the O.D. at 660 nm. Plot the standard graph with concentration on the X-axis and O.D. on the Y-axis. Check for the concentration of sample using the standard graph. Then calculate Phosphorus in mg/
100 g using the following formula.

**Calculation**

\[
P (\text{mg/100 g}) = 25 \times \text{Vol. made up in ml} \times (\text{Conc. in } \mu\text{g/ml}) \times \text{Dilution} \times 100 / \text{Wt of sample in gm} \times \text{Volume taken} \times 1000
\]

Where: 100 is for 100 g of food sample

1000 is for converting µg to mg.

**Analysis of Anti-oxidants:** Total phenolic content, Total flavonoid content, DPPH radical scavenging activity, ABTS antioxidant assay were determined according to the procedures described by Lakshmi et al. (2021).

**Estimation of Total phenolic content (TPC):**

Sample (0.5 g) was extracted with ethanol/distilled water (2 x 25 ml) with agitation for 3h each time, the supernatants were obtained by centrifugation at 5000 rpm for 10 min at 4 °C and the sample extract was used for further analysis. 200 µl of sample extracts were mixed with 2 ml of 10% Na₂CO₃ solution. Incubated at room temperature for 3 min, then 100 µl of Folim-Ciocalteu reagent was added to the mixture. The resulting solution was incubated for 90 min at room temperature under dark, the absorbance was measured at 765 nm using the UV-Vis Spectrophotometer. The TPC was expressed as gallic acid equivalent (GAE) in milligrams per 100 gram of sample.

**Estimation of Total flavonoid content (TFC):**

Sample (0.5g) was extracted with 80% methanol (2 x 25 ml) for 3 h. Sample extract (250 µl) was diluted with 1.25 ml distilled water. Sodium nitrite (75 µl of 5% solution) was added and the mixture was allowed to stand for 6 min. Further, 150 µl of a 10% aluminium chloride was added and the mixture was allowed to stand for 5 min. After that, 0.5 ml of 1 M sodium hydroxide was added and solution was mixed well. The absorbance was measured immediately at 510 nm using a spectrophotometer. Catechin was used as standard and the results were reported as milligram of catechin equivalent per 100 gram of sample.

**Estimation of DPPH radical scavenging activity:**

The sample (1.0 g) was extracted with methanol (2 x 25 ml) for 3 hour and centrifuged for 4000 rpm for 10 min. The supernatant was estimated for ABTS assay. The extract (100 µl) was reacted by adding 2.9 ml of ABTS+ reagent and the absorbance was determined after 30 min. The percentage inhibition of the sample extracts were evaluated using the formula,

\[
\text{ABTS assay (% inhibition)} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right) \times 100
\]

where \(A_{\text{control}}\) is the absorbance of the control reaction (containing all reagents except test compound) and \(A_{\text{sample}}\) is the absorbance of the test compound.

**Estimation of Ferric reducing antioxidant power assay:** Ferric reducing antioxidant power assay was estimated as per the procedure described by Benzie and Strain(1996). Briefly, the FRAP reagent was prepared from sodium acetate buffer (300 mmol/l, pH 3.6), 10 mmol/l TPTZ solution using 40 mmol/l HCl as solvent and 20 mmol/l Iron (III) chloride solution in a volume ratio of 10:1:1, respectively. The FRAP reagent was prepared freshly daily and warmed to 37 °C in a water bath prior to use. 100 ml of the sample extract was added to 3 mL of the FRAP reagent. The absorbance of the mixture was measured at 593 nm after 4 min. The standard curve was constructed using FeSO₄ solution, and the results were expressed as µmol Fe(II)/g dry weight (DW) of cereal grains.

**Estimation of Vitamin C:** vitamin C was determined using indophenol method as described by Nielsen, (2017), Pipette out 5 ml of the working standard solution into a 100 ml conical flask, Add 10 ml of 4% oxalic acid and titrate against the dye (V₁ ml). End point is the appearance of pink colour which persists for a few minutes. The amount of the dye consumed is equivalent to the amount of ascorbic acid. Extract the sample (0.5-5.0 g
depending on the sample) in 4% oxalic acid and make up to a known volume (100 ml) and centrifuge. Pipette out 5 mL of this supernatant, add 10 mL of 4% oxalic acid and titrate against the dye (V₂ ml).

Calculation: Amount of ascorbic acid mg/100g sample

\[
\frac{0.5 \text{mg}}{V_1 \text{mL}} \times \frac{V_2 \text{mL}}{5 \text{mL}} \times \frac{100 \text{mg}}{\text{Wt. of the sample}} \times 100
\]

Note: Acetic-metaphosphoric acid mixture may be used instead of 4% oxalic acid.

RESULTS AND DISCUSSION

Table 1. Proximate analysis of lemon peel powder: (g/100g)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Lemon peel Powder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>9.75±0.78</td>
</tr>
<tr>
<td>Protein (g/100g)</td>
<td>7.19±0.09</td>
</tr>
<tr>
<td>Fat (g/100g)</td>
<td>1.52±0.09</td>
</tr>
<tr>
<td>Ash (g/100g)</td>
<td>2.20±0.19</td>
</tr>
<tr>
<td>Crude fiber (g/100g)</td>
<td>15.11±0.12</td>
</tr>
<tr>
<td>Carbohydrate (g/100g)</td>
<td>48.14±0.47</td>
</tr>
</tbody>
</table>

The values shown represent the mean ± standard deviation.

Table 1 represents the Proximate analysis of lemon peel powder revealed the moisture was found to be 9.75 percent indicating that the lemon peel powder has relatively low water content, which is important for stability and shelf life. In terms of macronutrients, the powder contained 7.19 g of protein indicating a moderate protein content and the approximately similar protein content was reported by Janati et al., 2012 protein content was 9.42g/100g. This suggests that lemon peel powder could be a potential source of plant-based protein in various food products.

Furthermore, the analysis showed that the powder contained 1.52 g indicating a low-fat content. This makes it suitable ingredient for individual for individuals seeking low fat dietary options. The ash content, at 2.20 g indicates the presence of mineral elements in the lemon peel powder, which can contribute to its nutritional value. One notable finding was that the high content of crude fiber which measured 15.11 g the same fiber content was found by Janati et al. 2012. This suggests that lemon peel powder is a rich source of dietary fiber, which has various health benefits, including promoting digestive health and potentially aiding in weight management. Lastly the carbohydrate content was found by 48.14 g, making up a significant portion of the powder’s composition. This indicates that lemon peel powder is a carbohydrate-rich ingredient, which can provide energy and be utilized in various culinary applications. Similar proximate composition in lemon peel was reported by Pranav et al., 2017.

Table 2. Mineral composition of lemon peel powder:(mg/100g)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Lemon peel Powder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron (mg/100g)</td>
<td>7.73 ±0.00</td>
</tr>
<tr>
<td>Calcium (mg/100g)</td>
<td>401.58±3.18</td>
</tr>
<tr>
<td>Zinc (mg/100g)</td>
<td>8.21±0.08</td>
</tr>
<tr>
<td>Sodium (mg/100g)</td>
<td>146.39±2.61</td>
</tr>
<tr>
<td>Potassium (mg/100g)</td>
<td>511.60±11.47</td>
</tr>
<tr>
<td>Phosphorus (mg/100g)</td>
<td>818.59±11.48</td>
</tr>
</tbody>
</table>

The values shown represent the mean ± standard deviation.

Table 2 presents the mineral composition of lemon peel powder, providing essential information about the presence of various minerals in this dietary component. These minerals play vital roles in maintaining overall health and well-being. Firstly, the lemon peel powder was found to be rich in iron, containing 7.73 mg. Iron is a crucial mineral involved in the formation of red blood cells and the transportation of oxygen throughout the body. Its presence in lemon peel powder suggests that it can contribute to meeting daily iron requirements when incorporated into the diet.

The calcium content in the lemon peel powder was measured at 401.58 mg/100 g. Calcium is essential for bone and teeth health, as well as various physiological processes in the body. The presence of calcium in lemon peel powder suggests that it can be a valuable source of this mineral, particularly for individuals who may have dietary restrictions or preferences that limit their intake of dairy products.

The zinc content of lemon peel powder was 8.21 mg is also noteworthy. Zinc is essential for immune function, wound healing, and many enzymatic reactions in the body. Lemon peel powder can provide a significant contribution to daily zinc intake.

Sodium, at 146.39 mg, and potassium, at 511.60 mg, are important electrolytes that help maintain fluid balance and nerve function in the body. The sodium content in lemon peel powder is relatively low, which can be beneficial for individual monitoring their sodium intake. On the other hand, the high potassium content makes lemon peel...
powder potentially useful in promoting heart health and regulating blood pressure.

The phosphorus content in the lemon peel powder was found to be 818.59 mg. Phosphorus is essential for bone health, energy metabolism, and various cellular functions. The presence of phosphorus in lemon peel powder contributes to its overall nutritional value.

The values shown represent the mean ± standard deviation. TPC-total phenolic content, TFC-total flavonoid content, GAE-gallic acid equivalent, CE-catechin equivalent

The results presented in Table 3 reveal the remarkable antioxidant potential of lemon peel powder, shedding light on its significance as a valuable dietary and functional ingredient. The substantial vitamin C content of 60.37 mg underscores its role as an excellent source of this essential antioxidant vitamin, known for its ability to combat oxidative stress and support immune function. Moreover, the high level of total polyphenols (797.64 mg GAE/100g) and total flavonoids (639.57 mg CE/100g) in the lemon peel powder indicate its richness in these potent natural antioxidants. Similarly, studies reported that the higher amount of phenolic compounds and ascorbic acid are present in the lemon peel by John et al.2017. These compounds have linked to a range of health benefits, including reducing the risk of chronic diseases by neutralizing free radicals that can damage cells.

The DPPH and ABTS radical scavenging activities, with inhibition percentage of 49.56% and 38.77%, respectively, highlight the lemon peel powder’s ability to effectively counteract harmful free radicals. This antioxidant capacity can play a crucial role in protecting cells and tissues from oxidative damage, which is associated with various health issues. The ferric reducing antioxidant power of 3.19µmol Fe(II)/g further supports the notion that lemon peel powder possesses potent antioxidant properties, which can contribute to overall health and well-being. Similarly, (Jyoti et al) conducted the study on Use of Various Processing Technique with Citrus Fruits Peel to Analyze Nutritional, Physiochemical and Antioxidant Properties and found that the Ferric reducing ions content of the blanched lemon peel powder was 0.47%. This variation is due varietal and cultivation difference in lemon.

CONCLUSION

The evaluations of the proximate composition of lemon peel powder are valuable source of nutrients. The elemental analysis shown that it contains some nutrients required for normal functioning of the body system. It is a good source of total polyphenols and has good antioxidant properties which will make it a useful ingredient in the preparation of various food products. Overall, the results suggested that lemon peel waste could be used as a raw material for many products. From industrial point of view, fruit peel which is the residues from processing industry could be further processed for value addition of various food products.

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