Study of physicochemical and antioxidant synergy efficacy of betel leaf dried paste powder

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\begin{abstract}

The present study analyses the drying kinetics and changes in qualities during hot air drying of betel leaf paste for the manufacturing of betel leaf paste powder. A comparative study was conducted to evaluate the changes in different properties, including physico-chemical properties, proximate composition and other functional properties. The betel leaf paste dried powders have longer shelf life than raw leaves. Drying of betel leaf paste was conducted in three different levels, i.e., 50, 70 and 80 °C, till the samples reached a constant weight. The samples dried at 80 °C and 70 °C, showed the lowest water activity 0.1, whereas the sample dried at 50 °C, showed the highest water activity of 0.15. The values of protein, fat, crude fibre and ash were observed a little higher in betel leaf powder dried at 70 °C, than in other drying temperatures. This increase in the composition may be the result of higher dry matter in dried powder and less drying time of paste and minimum degradation in nutritional value at 70°C. However, the highest carbohydrate content was observed in betel leaf powder dried at 50 °C. The alcoholic extract of the oven-dried powder exhibited the DPPH radical scavenging activity (at 50 µg/mL) of 41.45, 46.32, and 44.35 at temperatures of 50, 70 and 80 °C respectively. The amount of phenolic content in the ethanolic extract was found to be 307, 322, and 316 mg/g in oven-dried betel leaf paste powder at 50, 70 and 80 °C respectively.

\textbf{Keywords:} betel leaf paste antioxidants drying water activity DPPH
\end{abstract}

Introduction

\textit{Piper betle}, belonging to the \textit{Piperaceae} family, widely known as betel leaf finds its wide use in different parts of the world, which is an auspicious mark since they can be used as a food ingredient. Betel leaf plant is perennial and is widely cultivated in India, Sri Lanka, Malaysia and other Southeast Asian countries. These leaves also find application in curing stomach disorders, concerns of ageing etc. Nevertheless, as the danger of synthetic drugs and food additives in the form of preservatives, colouring agents and antioxidants continues to increase, humanity is becoming increasingly aware of natural resources and their benefits and is researching natural herbs for their medicinal, antimicrobial and therapeutic potential (Sarma et al., 2018; Vernekar et al., 2019; Madhumita et al., 2019).

Betel leaves are considered as a cash crop with desirable nutritional, organoleptic and therapeutic properties. Betel leaves are nutritious and have insecticidal, antitumor (Gundala and Aneja, 2014), antioxidant (Jaiswal et al., 2014), neuroprotective (Chan and Wong, 2014), antimicrobial (Nouri and Nafchi, 2014), antidiabetic, anti-helminthic (Shah et al., 2016), and many other therapeutic properties. A number of biologically active compounds, such as hydroxychavicol, pipperbetol, chavicol, chavibetol, piporol A, methylpipperbetol and piporol, are also present in the leaves. A volatile oil known as betel leaf oil is a key component of the leaf (Kumari and Rao, 2015; Widawati and Riandi, 2015).
Betel leaves have a shorter shelf life, despite having a rich supply of micronutrients since they are highly perishable in nature. Drying and dehydration are the strategies used for avoiding degradation of the perishable raw material and reducing the expense of packing, handling, storing and shipping the material by making it dry strong. Green leafy vegetables can be used for the enrichment of food products since they are rich in essential micronutrients (Datta et al., 2015). The present study was planned to evaluate the drying kinetics and changes in properties occurring during hot air drying of Kariyale varieties of the betel leaf. This study aims to encourage the use of betel leaf powder, as a fortifying ingredient, in the production of nutraceuticals and functional foods.

Materials and methods

Raw materials

Kariyale variety of betel leaf was procured from a near village of Bengaluru, Karnataka (INDIA) and washed with distilled water to remove adhering dirt. Cleaned leaves were chopped into pieces. Chopped pieces of betel leaves were blanched at 70 °C hot water for 10 minutes to make them soft and suitable for further processing. Blanched leaves were processed for making the paste, using a domestic kitchen mixer-grinder machine (Bajaj GX 8, India), as shown in Fig.1. All analytical grade (AR) reagents, chemical and solvents used in the quantitative analysis were manufactured by, “SD FINE-CHEM Limited (SDFCL)”, Mumbai (India)”, and procured from the local registered supplier of Bengaluru (India).

Fig. 1. Paste making procedure of the betel leaf

Drying of paste

Drying of betel leaf paste was done using a hot air oven (Manufacturer: Bangalore Scientific & Industrial supplies, Bangalore, India), where the paste was kept in low height laboratory trays and spread uniformly in a thin layer. In a hot air oven, the known weight of betel leaf paste samples was kept for drying at different temperatures, i.e., 50 °C, 70 °C, and 80 °C, till the samples reached constant weight. The dried powder was stored in separate air-tight containers as a stock sample in a refrigerator until required for analyses (Nwinuka et al., 2005).

Physicochemical properties of the powder

Moisture content, water activity (a_w) and total solid content

The moisture content of the powder was determined by the gravimetric method using Equation 1, at 105 °C as per standard method AOAC (1995). The water activity meter (AQUALAB CX-2 of Pullman, Washington, USA) was used to measure the water activity of powder.

For determining the total solid (TS) content, 5 g of samples were dried at 105 °C for 3h.

\[
MC (% \text{ wet basis}) = \left(\frac{\text{Mi} - \text{Mf}}{\text{Mi}}\right) \times 100 \quad \text{Eq. (1)}
\]

Where,

MC is moisture content of sample on a wet basis (%),

Mi=initial weight of sample in gram before drying,

and,

Mf= final weight of sample in gram after drying

\[
\text{TS} (%) = \left(\frac{\text{Mf}}{\text{Mi}}\right) \times 100 \quad \text{Eq. (2)}
\]

Where,

TS is total solid content in %.

Proximate composition analysis

The nutritional composition of the sample which includes carbohydrate, crude protein, fat, total ash and fibre was determined by the standard method of analysis (AOAC 1995).

Determination of antioxidant activity

Preparation of betel leaf extracts powder solutions

About 100 g of betel leaf paste powder sample was exhaustively extracted with 95% ethanol using soxhlet
extraction apparatus for about 15 hours. The residue was concentrated using a Rota-vacuum evaporator to obtain a solvent-free extract and stored at -18 °C till further use.

**DPPH radical scavenging activity**

The free radical scavenging activity of extracts of betel leaf paste powder was measured by using 1,1-diphenyl-2-picrylhydrazil (DPPH). Briefly, 1 mL of 0.1mM solution of DPPH in methanol was added to 3 mL of methanol extract of paste powder at different doses (25-100μg). The powder was shaken strenuously and kept aside at room temperature for 30 min; then spectrophotometer was used at 517 nm for the measurement of absorbance. The highest suggested free radical scavenging activity of the reaction mixture was obtained at the decreased value of absorbance. The DPPH radical concentration was estimated using the following Equation 3, as given by Shrivastava et al. (2010):

\[
\text{DPPH scavenging effect (\%)} = \frac{(A_c - A_t)}{A_c} \times 100 \text{ Eq. (3)}
\]

Where,

\(A_c\) was the absorbance of the control reaction, and
\(A_t\) was the absorbance in the presence of sample

**Determination of total phenolic content (TPC)**

Total phenolic content in the extracts of paste powder was estimated using Folin-Ciocalteu’s reagent method (Praveen et al., 2012). The gallic acid standard curve was used to measure the phenolic content and was expressed as mg/g gallic acid equivalent using an equation derived from the calibration curve of gallic acid. The values were taken three times and the average value expressed in mg/g of dry extract.

**Results and discussion**

**Physicochemical analysis**

**Moisture content and drying characteristics**

The total moisture content in betel leaf paste was 87.44% (w.b.) and the moisture content of betel leaf dried powder was 6.25, 4.82, and 3.45% (d.b.) at 50, 70, and 80 °C, respectively. Betel leaf was dried till constant weight and the complete drying of betel leaf was observed at 50, 70, and 80 °C for 6 hours 30 minutes, 4 hours 15 minutes, and 3 hours 45 minutes, respectively.

**Water activity**

The water activity of food samples plays an important role in their shelf life. High water activity of food products may lead to the growth of microorganisms and spoilage. The water activity was obtained in oven 50, 70, and 80 °C and the water activity of dried powder was 0.15, 0.1, and 0.1 respectively. Oven-dried powder at 70 and 80 °C can be stored for a longer time due to the less observed water activity.

**Total solids (TS)**

The total solid content in paste was 11.29% and it was obtained by oven-drying.
Table 1. Proximate composition of betel leaf paste powder

<table>
<thead>
<tr>
<th>Drying Temperature (°C)</th>
<th>Moisture (%)</th>
<th>Protein (%)</th>
<th>Fat (%)</th>
<th>Ash (%)</th>
<th>Crude Fibre (%)</th>
<th>Carbohydrate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>6.25</td>
<td>15.22</td>
<td>5.52</td>
<td>15.66</td>
<td>6.80</td>
<td>48.80</td>
</tr>
<tr>
<td>70</td>
<td>4.82</td>
<td>16.90</td>
<td>7.21</td>
<td>16.88</td>
<td>9.23</td>
<td>44.22</td>
</tr>
<tr>
<td>80</td>
<td>3.45</td>
<td>16.74</td>
<td>6.18</td>
<td>16.25</td>
<td>7.45</td>
<td>45.42</td>
</tr>
</tbody>
</table>

**Nutritional analysis of the powder**

All the nutritional values are expressed on basis of the dry weight of powder. The value of protein, fat, crude fibre and total ash was higher in powder dried at 70 °C, since the shorter drying time and a higher percentage of total solid in paste powder dried at 80 °C compared to 70 °C and 50 °C, lead to the higher nutritional value. From Table 1, it can be observed that the carbohydrate content was highest for the sample which was dried at 50 °C, followed by sample dried at 80 °C and 70 °C. However, samples dried at 70 °C showed the highest protein, fat, ash and crude fibre content, and minimal content was observed for the samples which were dried at 80 °C and 50 °C respectively. Higher temperature drying at 80 °C may have resulted in the breakdown of protein and fat, resulting in lesser proximate composition. The trend was not the same for moisture content, as higher the drying temperature, the lower the moisture content. Hence, as shown in Table 1, the sample dried at 50 °C had the highest, whereas the sample dried at 80 °C had the lowest moisture content.

**Antioxidant activity**

**DPPH Radical scavenging activity**

The antioxidant activity was estimated through free radical scavenging of betel leaf paste powder extracts. DPPH radical scavenging activities of powder extracts were evaluated. Methanolic extracts of powder showed synergetic DPPH radical scavenging activity. The oven-dried betel leaf paste powder was extensively reported to have an antioxidant activity array of free radicals. The alcoholic extract of the oven-dried powder samples exhibited the DPPH radical scavenging activity (at 50 µg/mL) as 41.45, 46.32, and 44.35 at 50 °C, 70 °C, and 80 °C, respectively. The sample powder dried at 70 °C had the highest antioxidant activity and possibly less inactivation of polyphenol oxidase, as compared to the paste dried at 80 °C and 50 °C. Higher temperature drying of sample, at 80 °C and longer drying time at 50 °C, resulted in the degradation of the polyphenol oxidase, causing a decrease in the antioxidant activity.

**Determination of total phenolic content**

The Folin-Ciocalteu method was used to determine the ethanol extract of betel leaf paste powder to find the total phenolic content and the results were measured as equivalents of gallic acid. The amount of phenolic compound in the ethanolic extract was found to be 307, 322, and 316 mg/g in oven-dried powder at 50 °C, 70 °C, and 80 °C, respectively. As per research evidence, the phenolic compounds are the major antioxidant compounds that exhibit radical scavenging efficiency, and numerous reactive oxygen species are extensively distributed among all plant materials.

**Conclusions**

The dried betel leaf paste powder was found to exhibit concentrated nutrition and enhanced antioxidant properties. The lower water activity was observed in the dried powder, which can efficiently help to store for a longer time. The paste dried at 70 °C showed a higher amount of nutrition, total phenolic content and antioxidant activity as compared to the sample powder dried at 50 °C. Further increase in drying temperature, i.e., drying of the sample at 80 °C, resulted in lowering of nutritional and functional properties. The dried powder with increased nutritional properties and longer shelf life would be useful for pharmaceutical, traditional health system sectors and transportation.

**Author Contributions:** Chandan Kumar Sahu: Involved in experiment planning, experimentation, analysis and interpretation of data, drafting of the manuscript and critical revision; Angitha Balan: involved in study conception, experiment planning and data acquisition; Venkata Krishna Bayineni: involved in experimentation, data acquisition and drafting of the manuscript; Soumitra Banerjee: manuscript drafting and editing

**Funding:** This research received no external funding.

**Acknowledgements:** The authors would like to thank the Director, Centre for Incubation, Innovation, Research and Consultancy (CIIRC), Jyothi Institute of Technology, Bengaluru, Karnataka (India) and Prayoga Institute of Education Research (PIER), Bengaluru, Karnataka (India) for providing necessary financial, research and infrastructure support for the successful completion of this work.
Conflicts of Interest: The authors declare no conflict of interest.

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