Effect of ethyl oleate assisted blanching and drying temperature on antioxidant properties of Bitter gourd

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Abstract

Bitter gourd is an antioxidant and nutrient-rich vegetable in the Cucurbitaceae family. It is also rich in vitamin C, vitamin A, β-carotene, phosphorous and iron. It is an excellent source of both enzymatic and non-enzymatic antioxidant properties like 2, 2, diphenyl picrylhydrazyl, total phenolic compound and ferric reducing antioxidant power. The conventional blanching method reduces the antioxidant components and other bioactive compounds during processing. This paper explores the effects of ethyl oleate assisted blanching on antioxidant properties of bitter gourd dried at three different temperatures of 50, 60 and 70ºC. Retention of vitamin C, total phenolic compounds, 2,2, diphenyl picryl hydrazyl was highest for combined pre-treatment of ethyl oleate and microwave blanched samples.

Keywords: bitter gourd, conventional blanching, antioxidant, combined ethyl oleate and microwave
1. Introduction

Bitter gourd (*Momordica Charantia L.*) is a known vegetable belongs to *Cucurbitaceae* family mostly consumed in Africa, South America and South Asia. It is commonly known as bitter melon (English), karela (Hindi), pakar (Tamil) and karavelli (Sanskri) [1]. The bitter nature of this vegetable helps to prevent diabetes, cancer, cholesterol and other digestive disorders [2]. It is rich in vitamins (C, A, E, B1, B2, B3 and B9) and minerals (potassium, iron, magnesium, calcium, phosphorous and zinc). The natural presence of phenolic and bioactive components are responsible for its medicinal properties like antidiabetic, anticancer, antitumor, antifertility, antimicrobial, antiviral and antioxidant activity [3] [4]. Momordicin and charantin are the two principal components cause bitterness [5]. Bitter gourd has higher scavenging activity (82%) and ferric reducing antioxidant power (FRAP) than any other vegetable [6]. Various thermal processing methods like water blanching, microwave blanching were adopted in order to increase the antioxidant properties of vegetables. The application of heat during processing releases the phenolic compounds from the plant matrix thus increases the total phenolic compounds [7]. Water blanching and boiling methods highly increased the total phenolic compounds and radical scavenging activity in bitter gourd [8] [9]. However, degradation of water soluble vitamins due to leaching was the major limitation in hot water blanching. Few researches suggested that application of microwave highly increased the nutrient and other bioactive components in vegetables [10]. Microwave pre-treatment significantly increased the ascorbic acid, radical scavenging activity, total phenolic, chlorophyll, carotenoids in Brussel [11], bitter gourd [12], dill and parsley leaves [13]. Later, it was investigated that ethyl oleate increased the beta carotene and inhibited the formation of 5-hydroxymethylfurfural (HMF) during drying of strawberry tree [14]. But there is not much research available on effect of ethyl oleate on antioxidant properties of vegetables. Drying plays an important role in processing and value addition. Drying with appropriate pre-treatment could help to retain the antioxidant properties and other sensory attributes. The main objective of this research is to study the combined effect of ethyl oleate and blanching on antioxidant properties of bitter gourd dried at different temperatures.
2. Materials and methods

2.1. Raw material

Fresh bitter gourds were procured from local vegetable market. These were then washed and packed in polyethylene pouches and stored at 4°C until used.

2.2. Pre-treatments and drying

Bitter gourds were sliced into 3.5 cm dia and 0.5 cm thick using stainless steel knife. Seeds and pith were removed. Pre-treatments (Table 1) like ethyl oleate treated water blanching (EOWB) and ethyl oleate treated microwave blanching (EOMB) were given to sliced bitter gourds. Bitter gourd without any pre-treatment was taken as control. All the pre-treated and control samples were dried in tray dryer at three different temperatures (50, 60 and 70°C) maintaining at an air velocity of 0.8 m/s. For every 5 minutes’ interval the moisture loss was taken using electronic weighing balance (Model No: IND/09/08/558, WENSOR, India). When the sample reached the constant weight, drying was stopped and dried bitter gourds were then packed in low density polyethylene (LDPE) pouches and stored in desiccator for further analysis.

Table 1 Pre-Treatments and its Methodology

<table>
<thead>
<tr>
<th>Pre-treatments</th>
<th>Methodology</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOP</td>
<td>Dried without any pre-treatment</td>
</tr>
<tr>
<td>EOWB</td>
<td>Dipped in 2% EO and 4 %K₂CO₃ for 1 min and water blanched at 80°C for 3 minutes</td>
</tr>
<tr>
<td>EOMB</td>
<td>Dipped in 2% EO and 4 %K₂CO₃ for 1 min and microwave blanched at 600W for 135 seconds</td>
</tr>
</tbody>
</table>

2.3. Sample extraction

Dried sample (1g) was finely grinded in mortar and pestle by adding 20-30 ml of acetone. This extract was then homogenised in magnetic stirrer for 1 hour. After
complete homogenisation, the mixture was centrifuged at 3000 rpm for 20 mins. The aliquot collected used for analysis [15]

2.4. Vitamin C

Vitamin C is an important nutrient in the human diet, but is easily reduced or destroyed by exposure to heat and oxygen during processing, packaging, and storage of food. Vitamin C was determined by Eq. 1 given by [16]

\[ \text{mg of AA/ml} = (X - B) \times \frac{F}{E} \times \frac{V}{Y} \]  \hspace{1cm} (1)

where, \(X=\) titre value of sample, \(B=\) titre value of blank, \(F=\) titre value of dye standardisation, \(E=\) amount of sample taken, \(V=\) dilution factor, \(Y=\) ml of sample taken for titration

2.5. Total phenolic content (TPC)

Total phenolic compound was determined with a slight modification [15]. An aliquot of 0.5 ml was added with 2 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagent. The mixture was left for 5 mins before adding 2ml of 7.5% sodium carbonate and kept in a dark room. After 1 hour, the absorbance was taken in UV-vis spectrophotometer (Dynamica, Australia) at 765 nm. The calibration curve of gallic acid was used as standard at 0.2, 0.4, 0.6, 0.8 and 1 with \(R^2 = 0.9968\). The TPC results were expressed in mg of GAE/100 g.

2.6. 2,2, Diphenyl Picryl Hydroxyl (DPPH)

DPPH scavenging activity of bitter gourd was determined using Eq.2. [17]. A stock solution of DPPH was prepared by dissolving 4 mg in 100 ml methanol and stored at -20°C until used. A working standard was prepared by mixing 35 ml of stock solution with 35 ml of methanol and absorbance was observed at 516 nm using a UV-Vis spectrophotometer. 100 µL of bitter gourd extract was mixed with 1.5 ml of methanolic DPPH and kept overnight in the dark and absorbance was taken at 516 nm.

\[ \text{DPPH scavenging activity (\%)} = \frac{(\text{blank } A - \text{ sample } A)}{(\text{blank } A)} \times 100 \] \hspace{1cm} (2)

Where A-Absorbance
2.7. **Ferric Reducing Antioxidant Power (FRAP)**

FRAP working reagent was prepared using 300mM acetate buffer (3.1 g sodium acetate trihydrate, 16 ml glacial acetic acid made up to 1:1 with distilled water, pH 3.6 ), 10 mM 2,4,6-tris (2-pyridyl)-s-triazine (TPTZ) in 40 mM HCl and 20 mM ferric chloride (FeCl₃·6H₂O) in the ratio of 10:1:1. About 50 µL of bitter gourd extract was mixed with 0.5 ml of FRAP reagent and absorbance was taken at 595 nm after 30 minutes. The standard curve (R²=0.9798)of Trolox was calibrated to determine the activity capacity of samples. The results were expressed as Trolox equivalent per 100 g of dried bitter gourd (mg of TAE/100 g of DW)

2.8. **Statistical analysis**

All data were analysed using statistically using Statistical Package for the Social Sciences (SPSS-21). Statistical difference was determined by Tukey’s method and significant level used for all data was P≤0.05.

3. Results and discussion

3.1. **Effect of pretreatment and drying temperature on vitamin C**

The combined effect of pre-treatment and temperature on vitamin c is given in Table 2. Fresh bitter gourd was found to possess higher (94 mg/100g) vitamin C [8]. Pre-treatments significantly (P<0.05) retained the Vitamin C of dried sample. The combined ethyl oleate and microwave blanched samples retained the maximum vitamin C (84.34 mg/100g) whereas not pretreated samples retained 64.69 mg/100g at 50°C. The temperature significantly(P<0.05) affected the vitamin C. As the temperature increased from 50 to 70°C, vitamin
C was reduced from 64.69 to 41.61 mg/100g which is similar to the results that are reported in orange [18].

Table 2 Effect of pre-treatment and temperature on Vitamin C

<table>
<thead>
<tr>
<th>Drying temperature</th>
<th>NOP</th>
<th>EOWB</th>
<th>EOMB</th>
</tr>
</thead>
<tbody>
<tr>
<td>50°C</td>
<td>64.69±0.36 aA</td>
<td>74.16±0.15 bA</td>
<td>84.34±0.17 cA</td>
</tr>
<tr>
<td>60°C</td>
<td>54.02±0.06 aB</td>
<td>71.14±0.09 bB</td>
<td>80.14±0.09 cD</td>
</tr>
<tr>
<td>70°C</td>
<td>41.61±0.05 aC</td>
<td>65.25±0.15 bC</td>
<td>74.25±0.56 cC</td>
</tr>
</tbody>
</table>

* different small letters in each column indicates significant (p<0.05) effect of pre-treatments
* different capital letters in each row indicates significant (p<0.05) effect of temperature

3.2. Effect of pre-treatment and drying temperature on TPC

Total phenolic compound of fresh bitter gourd was found to be 6.89 mg of GAE/100g which is higher [19] i.e 5.92 mg/100g for bitter gourd. The effect of pre-treatments at varied drying temperatue is given in Table 3. The effect of ethyl oleate and subsequent blanching (hot water and microwave) significantly (P<0.05) increased the TPC. The highest TPC (33.03 mg of GAE/100g) was found in ethyl oleate treated and microwave blanched sample at 60°C whereas not pre-treated sample was 9.87 mg of GAE/100g. The drying temperature had significant (P<0.05) effect on TPC. The increase in temperature from 50 to 60°C increased the TPC from 9.87 to 21.34 mg of GAE/100g. It is also reported that the increase in temperature breaks down the cellular constituents and thus releasing the more phenolic compounds from the sample [12]. However, the further increase in
temperature decreased the TPC for all samples. These results were matched with Tan et al. and the TPC was resulted at 40, 50 and 60°C were 1533, 1925 and 1847 mg of GAE/100 g respectively [15]. This is due to the processing in higher temperature releases the hydrolytic and oxidative enzymes and thus destroying the antioxidant activity [20].

### Table 3 Effect of pre-treatment and temperature on TPC

<table>
<thead>
<tr>
<th>Drying temperature</th>
<th>NOP</th>
<th>EOWB</th>
<th>EOMB</th>
</tr>
</thead>
<tbody>
<tr>
<td>50°C</td>
<td>9.87±0.03</td>
<td>13.93±0.54</td>
<td>29.67±0.39</td>
</tr>
<tr>
<td>60°C</td>
<td>21.34±0.17</td>
<td>22.18±0.09</td>
<td>33.03±0.27</td>
</tr>
<tr>
<td>70°C</td>
<td>7.35±0.17</td>
<td>8.47±0.15</td>
<td>9.59±0.05</td>
</tr>
</tbody>
</table>

* different small letters in each column indicates significant (p<0.05) effect of pre-treatments
* different capital letters in each row indicates significant (p<0.05) effect of temperature

3.3. **Effect of pre-treatment and drying temperature on DPPH**

The effect of pre-treatments and temperature on DPPH is given in Table 4. The fresh bitter gourd had 85.98 % of scavenging activity which is higher that those reported by Hamissou et al. ie. 82.05 % [21]. The pre-treatments significantly (P<0.0.5) affected the DPPH of dried bitter gourd. Ethyl oleate treated water blanching reduced the DDPH activity due to leaching at higher temperature [22]. But, ethyl oleate treated microwave blanching retained the higher antioxidant activity 81.39 % whereas not pre-treated sample reatined 80.12% at 50°C. The temperature had significant effect on DPPH scavenging activity. The
increase in temperature from 50 to 70ºC decreased the antioxidant activity from 80.12 to 75.44 % respectively. These results were matched with Tan et al. [15]. There is no significant (P>0.05) difference of DDPH found between 50 and 60ºC.

**Table 4** Effect of pre-treatment and temperature on DPPH

<table>
<thead>
<tr>
<th>Drying temperature</th>
<th>NOP</th>
<th>EOWB</th>
<th>EOMB</th>
</tr>
</thead>
<tbody>
<tr>
<td>50ºC</td>
<td>80.12±0.09 bB</td>
<td>74.76±0.12 aB</td>
<td>81.39±0.19 cB</td>
</tr>
<tr>
<td>60ºC</td>
<td>78.44±0.02 bB</td>
<td>77.28±0.05 aB</td>
<td>80.38±0.08 cB</td>
</tr>
<tr>
<td>70ºC</td>
<td>75.41±0.21 bA</td>
<td>71.39±0.21 aA</td>
<td>79.98±0.09 cA</td>
</tr>
</tbody>
</table>

* different small letters in each column indicates significant (p<0.05) effect of pre-treatments
* different capital letters in each row indicates significant (p<0.05) effect of temperature
* same capital letters in row indicates no significant (p>0.05) effect

**3.4. Effect of pre-treatment and drying temperature on FRAP**

The combined effect of pre-treatment and temperature on FRAP is given in Table 5. FRAP value for fresh bitter gourd was found 5233.59 mg of Trolox/100g of DW which is lower than those reported by Tan et al [15]. The pre-treatments significantly (P<0.05) reduced the FRAP activity of bitter gourd. Not pre-treated sample had highest FRAP (4945.33 mg of Trolox /100g of DW) followed by EOMB (512.28 mg of Trolox /100g of DW) and EOWB (450.87 mg of Trolox /100g of DW) at 50ºC. however, EOMB retained the maximum FRAP (2810.52 mg of Trolox /100g of DW) dried at 70ºC due to the short exposure of drying time. These results were matched with Aminah and Permatasari [12] who reported that microwaving pre-treatment significantly retained the higher FRAP values at higher temperature. FRAP value was significantly (P<0.05) affected by temperature. [23]The increase in temperature decreased the FRAP value for not pre-treated samples from 4945.53 to 4073.68 mg of Trolox/ 100g of DW whereas
the FRAP value increased from 512.28 to 2810.52 mg of Trolox/100g of DW for EOMB treated samples. Thus the effect of ethyl oleate and microwave gave positive effect on FRAP during drying at higher temperature.

Table 5 Effect of pre-treatment and temperature on FRAP

<table>
<thead>
<tr>
<th>Drying temperature</th>
<th>NOP</th>
<th>EOWB</th>
<th>EOMB</th>
</tr>
</thead>
<tbody>
<tr>
<td>50°C</td>
<td>4945.33±0.07&lt;sup&gt;cA&lt;/sup&gt;</td>
<td>450.87±0.11&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>512.28±0.17&lt;sup&gt;bA&lt;/sup&gt;</td>
</tr>
<tr>
<td>60°C</td>
<td>4843.21±0.12&lt;sup&gt;cB&lt;/sup&gt;</td>
<td>1687.71±0.08&lt;sup&gt;aB&lt;/sup&gt;</td>
<td>1722.80±0.18&lt;sup&gt;bB&lt;/sup&gt;</td>
</tr>
<tr>
<td>70°C</td>
<td>4073.68±0.24&lt;sup&gt;cC&lt;/sup&gt;</td>
<td>2617.54±0.09&lt;sup&gt;aC&lt;/sup&gt;</td>
<td>2810.52±0.10&lt;sup&gt;bC&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* Different small letters in each column indicate significant (p<0.05) effect of pre-treatments.
* Different capital letters in each row indicate significant (p<0.05) effect of temperature.

4. Conclusion

Pre-treatments like hot water blanching and microwave blanching helps to increase the shelflife of perishables by reducing the concentration of enzymes and microbial load. However, these methods have their own merits and demerits. The combined effect of ethyl oleate and blanching could be an alternative blanching method to overcome these drawbacks. Ethyl oleate treated microwave blanching significantly increased the antioxidant properties of Bitter gourd with minimal leaching loss. Further, research is needed to study the effect of various concentration of ethyl oleate on quality attributes of other antioxidant rich vegetables.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References


