Optimization of extraction of bitter gourd (Momordica charantia L.) extract

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OPTIMIZATION OF EXTRACTION OF BITTER GOURD
(Momordica charantia L.) EXTRACT

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ABSTRACT

The objective of the present work is to identify an appropriate extraction method for bitter gourd with maximum retention of active components in the bitter gourd extract. The crushed bitter gourd extract, the homogenized crushed bitter gourd extract (homogenate), obtained by High pressure homogenizer at 300 psi and filtrate obtained by cloth filtration are compared by studying the phyto-chemical screening and colour value. The particle size distribution is also determined for crushed bitter gourd extract and homogenate samples. The results shown that homogenate sample has reduced particle size, more active agents and increased colour value. This is because most of solid components get retained in cloth filter but in homogenate, particle size of the solid components is reduced by cell disruption caused by high pressure thereby forming micro sized bitter gourd extract, so the homogenized bitter gourd extract can be used for preparing bitter gourd juice and powder by spray drying or freeze drying which provides enhanced shelf life and also maintaining the nutritive value and medicinal properties.

Keywords: High pressure homogenization, particle size distribution, momordin, charantin, phyto-chemical screening.

INTRODUCTION

Momordica charantia L. commonly known as bitter melon or bitter gourd which is tropical and subtropical climber of the family ‘Cucurbitaceae’. It is widely distributed in China, Malaysia, India and tropical Africa. Bitter gourd contains an array of biologically active plant chemicals including triterpenes, proteins, steroids, alkaloids, saponins, flavonoids and acids due to which plant possesses anti-fungal, anti-bacterial, anti-parasitic, anti-viral, anti-fertility, anti-tumorous, hypoglycemic and anti-carcinogenic properties (Beloin et al 2005; Grover and Yadav 2004; Scartezzini and Speroni 2000; Zafar and Neerja 1991). The taste of the bitter gourd is very bitter and most people could not bear its bitterness. However, despite of its bitter taste, bitter gourd has long been known for its anti-diabetic effect and much research was done to prove this. Bitter gourd contains a bitter compound called momordin-triterpene (Taylor 2002) and
charantin-alkaloid, insulin like peptides and a mixture of steroidal saponins (Fig. 1). It has been shown that these agents lower blood glucose level in rabbits (Lolitkar and Rao 1996; Tiroutchelvame et al., 2015).

![Fig. 1: Structure of Momordicin and Charantin](image)

Handling fluids under high pressure (up to 1500 psi) in continuous full-scale operation, known as homogenization is a common technology in food industry. The high pressure homogenization technology consists of pressurizing a fluid to flow through a narrow gap valve, which greatly increases its velocity, resulting in depressurization with consequent cavitation and high shear stress. Thus the macromolecules and suspended particles in the fluid (as cells and its fragments) are subjected to high mechanical stress, which are twisted (Fig. 2) and deformed (Floury et al 2004 and Pinho et al 2011). This technology has been studied by many authors as a non-thermal food preservation technique, especially for fruit and vegetable products, (Corbo et al 2010). It provides superior product stability, shelf life, digestion, and taste. Homogenizing can also considerably decrease the quantity of additives required. It makes feeds so that following spray drying or freeze drying produces the highest quality of powders. This is especially important for baby foods and many dairy and food products. Most commonly fat globules in milk are reduced to extremely small particles and distributing it uniformly throughout a fluid. But the use of homogenization for vegetables or fruit extract is uncommon. Hence, with this information, this research work is carried out to identify the appropriate extraction method for extraction of bitter gourd extract.

![Fig 2: Homogenization](image)
MATERIALS AND METHODS

Chemicals
Chloroform, sulphuric acid, potassium permanganate, Mayer’s reagent, sodium hydroxide, Benedict’s reagent, ferric chloride solution, ethanol and distilled water.

Raw material
Bitter gourd (M. charantia L.) was purchased from local markets of Chennai, India. The vegetable was brought to the laboratory and rinsed with water, surface sterilized and refrigerated for further processing.

Preparation of bitter gourd extract
The process started with selection of bitter gourd which was visually green in colour and absence of yellow colour, hardness and overall condition of bitter gourd (no bruise). The vegetable was washed, peeled and seeds were removed, sliced into small pieces. The bitter gourd pieces were subjected for grinding using a domestic mixer along with addition of an appropriate amount of distilled water to make bitter gourd extract.

The prepared bitter gourd extract was divided into three portions of which one part was subjected to high pressure homogenization at 300 psi, the second part was taken as such like crushed bitter gourd extract and the last part was subjected to cloth filtration using muslin cloth to prepare bitter gourd extract by filtering the solid particles. The resulting liquid phase is the filtrate sample. The quality analyses were carried out for homogenate sample, crushed bitter gourd extract and the filtrate sample.

Phyto-chemical screening
2ml of homogenate bitter gourd sample, crushed bitter gourd sample and filtrate sample were taken separately for preliminary phyto-chemical examination (Harborne 1989) as follows.

Test for alkaloids
The samples were boiled with dilute hydrochloric acid and filtered. The filtrate was tested with Mayer’s reagent which resulting the furnished alkaloid precipitates, indicating the presence of alkaloids in the samples.

Test for reducing sugar
To the sample, dilute sodium hydroxide solution was added followed by Benedict's solution, resulting brick-red precipitate, indicating the presence of a reducing sugar.

Test for tannins
To the samples a few drops of ferric chloride test solution were added, a deep blue colour was produced indicating the presence of tannins.

Test for charantin
Decolourisation takes place when samples were added to dilute potassium permanganate.

Test for terpenoids
To the samples, 2 ml of chloroform was added and 3 ml of con. H₂SO₄ was then added to form a layer. A reddishbrown precipitate colouration at the interface formed indicates the presence of terpenoids.

Test for flavonoids
To the samples few drops of 20% sodium hydroxide solution were added. A change to yellow colour which on addition of acid changed to colourless solution depicted the presence of flavonoids.

**Test for carotenoids**

To the samples, 10 ml of chloroform was added in a test tube and mixed thoroughly. The resulting mixture was filtered and 85% sulphuric acid was added. A blue colour at the interface showed the presence of carotenoids.

**Colour**

The colour analysis was carried out using Hunter lab Color Quest (Model: XE 3399) in terms of L, a* and b* values, where L indicates lightness, a* indicates redness (green to red) and b* indicates yellowness (blue to yellow). The average of the triplicate colour values of the samples were recorded after standardizing the equipment with a green tile as standard.

**Determination of particle size**

The particle size distribution of crushed bitter gourd extract and homogenized bitter gourd extract was carried out by laser scattering particle size distribution analyzer, Horiba LA950. The samples were loaded and the refractive index was kept at 1.4. Each sample was analyzed in triplicate and the data were reported as an average.

**RESULTS AND DISCUSSION**

**Phyto-chemical Screening**

The preliminary phyto-chemical screening was carried out for all bitter gourd samples. The test indicated that the homogenate and crushed bitter gourd samples showed the presence of alkaloids, reducing sugar, tannins, charantin, terpenoids, flavonoids, carotenoids, but the filtrate sample showed the absence of alkaloids, reducing sugar and flavonoids as shown in Table 1. It may be due to most of the solid particles such as bitter gourd tissue flakes got retained in the cloth and only the liquid phase was present in the filtrate, while in homogenate, there was no loss of solid particles and the whole crushed bitter gourd was converted to micro sized bitter gourd extract.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Observation</th>
<th>Homogenate</th>
<th>Crushed extract</th>
<th>Filtrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Alkaloid precipitate</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Reducing sugar</td>
<td>Brick red precipitate</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>Deep blue colour</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Charantin</td>
<td>Decolourisation</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Reddish brown precipitate</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Colourless solution</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Carotenoids</td>
<td>Blue colour interface</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*”+” indicates the presence of phytonutrient
*”-” indicates the absence of phytonutrient
Colour
The colour value of the homogenate was close to crushed bitter gourd extract (Table 2), but the filtrate sample was having difference in all parameters L, a*, b*. This may be because of the solid tissue and suspended cells that were retained in the cloth filtration, which are responsible for holding colour pigments in bitter gourd.

Table 2. Colour analysis of bitter gourd extract

<table>
<thead>
<tr>
<th>Sample</th>
<th>L</th>
<th>a*</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crushed bitter gourd extract</td>
<td>58.30 ± 0.05</td>
<td>4.95± 0.07</td>
<td>21.25± 0.05</td>
</tr>
<tr>
<td>Homogenate</td>
<td>59.89± 0.12</td>
<td>5.27± 0.04</td>
<td>20.18± 0.08</td>
</tr>
<tr>
<td>Filtrate</td>
<td>53.38± 0.07</td>
<td>5.12± 0.09</td>
<td>16.68± 0.05</td>
</tr>
</tbody>
</table>

Particle size
The particle size distribution of the crushed bitter gourd extract (non-homogenate) and homogenized bitter gourd extract was shown in Fig. 3 and Fig. The average mean diameter of the non-homogenate bitter gourd extract and homogenized bitter gourd extract was 492.65 and 146.63µm respectively. As expected the homogenization process has reduced the mean particle diameter of the bitter gourd extract

The reduction in the volume based particle mean diameter due to the homogenization process was reported in Fig.5. Three variations, diameter of 10%, 50% and 90% volume of both crushed bitter gourd extract and homogenized bitter gourd extract showed that there was gradual decrease in particle size distribution in all volumes.

![Fig.3. Particle size distribution of crushed bitter gourd extract](image)
Fig. 4. Particle size distribution of homogenized bitter gourd extract

Fig. 5: Effect of homogenization on mean particle size-volume based bitter gourd extract

CONCLUSION
The present work evaluated the effect of high pressure homogenization on the physico-chemical properties of bitter gourd extract. High pressure homogenization changed the particle size distribution, presence of phytonutrients and colour of the bitter gourd extract due to disruption of suspended particles or tissues in the bitter gourd extract. The laser light scattering images confirmed the particle size distribution in homogenate and crushed bitter gourd extract. The colour and phyto-chemical results were also shown the difference between homogenate, crushed bitter gourd extract and filtrate. These results inferred that the optimization of bitter gourd extract by homogenization could be used for preparing bitter gourd juice/powder by spray
drying or freeze drying which provides enhanced shelf life and also maintaining the nutritive value and medicinal properties.

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