Comparative evaluation on effect of drying methods on physicochemical characterization of wild bitter gourds Momordica charantia var. Muricata

Aabhishek AS, Archana Singh, Chidanand DV, Sunil CK and Bhagwat Madhura

DOI: https://doi.org/10.22271/chemi.2021.v9.i1ab.11508

Abstract
Momordica charantia var. muricata has shown a higher rate of antibacterial properties due to presence of specific secondary metabolites. In this paper, we have used an indigenously built convectional drying system that unlike other available systems does not depend on hot air rather a different approach altogether. The physicochemical characterization is compared amongst vacuum-assisted convectional drying, freeze drying and microwave drying, using instrumental analysis and wet-chemistry. The pharmacological efficacy of wild bitter gourd (WBG) in treatment of various ailments and medical conditions inclusive of diabetes mellitus, is a research gap with limited in-vivo studies available. WBG has shown effectiveness against inoculums of gram positive and gram-negative bacteria even post this drying technique. The dried WBG showed appreciable antioxidant activity (DPPH) making it a viable option to be utilized as an ingredient in production of fried products to extend the rancidity period. The evaluation of physical properties including color, water activity, shrinkage ratio and rehydration ratio have been discussed. Retention of total phenolic content (TPC), chlorophyll and ascorbic acid, post drying was evaluated. The research aims to explore chemical potency and changes in physicochemical parameters of WBG upon different drying techniques.

Practical Application: Since the medicinal value is higher; making it available for commercial production of nutraceuticals and pharmaceutical drugs, the product requires to be dried and powdered, and the nutritive composition needs to be evaluated. Utilization as ingredient in production of fried products to extend the rancidity period. The retention of certain nutritive compounds in wild bitter gourd powder (WBGP) can be utilized as value added product and as ingredient in development of functional foods and beverages based on its medicinal properties.

Keywords: Wild bitter gourd, drying, rehydration ratio, phenolics, antioxidant activity

1. Introduction
Momordica Charantia popularly acknowledged as bitter gourd belonging to the family cucurbitaceae, exhibits major therapeutic potential and is an active pharmaceutical ingredient owing to the presence of charantin (steroidal saponin) in them. According to ICAR- Indian Institute of Horticultural Research (IIHR), the particular bitter gourd variety of the study was identified as – ‘Arka Harit IIHR-4’, which produces yield of 12 tonne/hectare upon cultivation. It is known by several local names in different regions of India such as ‘Jhau Karela’ in Northern and Central India; while in Tamil Nadu it goes by ‘Chinna Pavakkai’. Wild bitter gourd (WBG) is especially grown in summer as well as rainy season and possess shorter storage periods due to excess moisture content. Generally among the major cash crops in India, two distinguished taxonomic varieties are cultivated in tropical region, Momordica charantia variety charantia (MCC), which possess elongated fruit size, while Momordica charantia variety muricata (MCM) exhibits small fruit size(Chakravarty 1990) [6] (Rai, Pandey, and Kumar 2008) [23]. Taxonomical difference among the two varieties is reported which shows greater chromosome size in MCM (1.32μm– 3.24μm) than MCC (1.27μm–3.07μm) (Ghosh, Bhowmick, and Jha 2018) [8]. WBGs include a mixture of steroidal saponins known as charantins, insulin-like peptides, and alkaloids. The phytochemical momordin has been clinically proven to inhibit cell division (cytotoxic activity) against in vivo lymphoma of...
Hodgkin and several additional *in vivo* studies showed the cytostatic and antitumor role of the whole bitter gourd plant (Paul and Raychaudhuri 2010) [22]. Various *in vitro* studies have also shown bitter melon’s anti-cancer and anti-leukemic activity against various cell lines including hepatic cancer, human leukemia, melanoma, and strong sarcomas (Bai *et al.* 2016) [4]. In addition to these properties, bitter melon leaf extracts have clinically demonstrated a strong antimicrobial activity across the spectrum. Antibacterial activity against *E. Coli*, *Pseudomonas*, *Staphylococcus*, *Salmonella*, *Streptobacillus* and *Streptococcus* are demonstrated *in vitro* by various leaf extracts of water, ethanol and methanol (Carini 2019) [5].

Due to SARS-CoV-2 and the lockdowns, sedentary lifestyle and increasing obesity has caught up with majority of the global population, which would further set a precedent to Type-2 Diabetes (T2D) (Holman *et al.* 2020) [12]. Tackling the concern against diabetes mellitus and breast cancer, wild bitter gourd has pharmacologically asserted hypoglycemic attributes (reducing of blood sugar) and other potentially beneficial actions (Carini 2019) [5] (Bai *et al.* 2016) [4].

Increased usage of designer drugs poses a major risk for public health by affecting the central nervous system (Weinstein *et al.* 2017) [29]. The globe is witnessing a good portion of population opting for natural alternatives that are plant derived over antibiotics, with increased risk factor involving the synthetic drugs (Savaio 2012) [24]. WBG includes a range of novel and bioactive phytochemicals, inclusive of steroids and triterpenes. Further research is needed to explore the functional properties of wild bitter gourd (WBG) and evaluate its absolute potency. Thus, the objective of present study is to evaluate and compare the product quality of dehydrated and pulverized WBG post drying in Microwave drying, Vacuum assisted Conventional drying, Freeze drying.
2.3.2 Microwave Drying
The prepared samples 250 ± 2.54g were arranged in a shallow microwavable dish covered with glass lid in a lab-scale single mode MW oven for 10 minutes. The drying was conducted at an output magnetron (vacuum tube with a central electron emitting cathode of highly negative potential surrounded by a structured anode) power of 360W (Fig 1b). The final moisture content was noted around 6% (w.b).

2.3.3 Freeze Drying
The prepared samples 250 ± 2.52g were previously frozen at -20°C for 24h in the deep freezer and then freeze dried using a lab-scale table top Freeze drier at -80°C (Fig 1a). The drying lasted until the final moisture content was reduced to approximately 6% (w.b).

2.4 Physical Tests
2.4.1 Moisture Content
Sample of known weight was taken and heated at 120°C using an IR moisture meter, until a constant weight (±0.02 g) was achieved. The moisture content was calculated using:

\[
\text{Moisture Content (\%)} = \frac{W - D}{W} \times 100
\]

where, W - Wet weight and D - Weight after drying

2.4.2 Color Measurement
Colour was analysed using Hunter Colour Lab (Hunter Association Laboratory, Inc., USA). The equipment was calibrated by placing the white and black plate prior to measurement. Fresh WBG samples were placed in a transparent glass holder and covered with lid, and subsequently the data was recorded. Dried WBG were analysed and data was recorded in similar manner. The colour was estimated for ‘L’, ‘a’ and ‘b’ values indicating the chromatic property of WBG. The total colour difference was calculated using:

\[
\Delta E = ((L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2)^{0.5}
\]

where, 
\( \Delta E \) = total color difference
\( L^*, a^*, b^* \) = color values of the sample
\( L_0^*, a_0^*, b_0^* \) = color values of reference material

2.4.3 Water Activity
Water activity (a\(_w\)) was determined using Aqualab Water Activity meter 4TE, maintaining measurement temperature at 24.09 ± 0.5 °C. Water activity was measured by placing fresh WBG followed by dried WBG, in a disposable cup in the water activity meter and sealing the sample chamber lid over the sample and waiting for vapor equilibrium.

2.4.5 Rehydration ratio
Dried samples of known weight were immersed in distilled water at 70 °C, and the weight of the samples were noted after 15min, 30min and 45min. The surface moisture was withdrawn using a dry blotting paper after taking out from the bath, and the change in weight was then recorded. The Rehydration Ratio was determined according to the equation below:

\[
RR = \frac{W_1}{W_2}
\]

where W1 and W2 are the mass values of the rehydrated and dried samples (g), respectively.

2.5 Chemical Tests
The dried WBG samples were pulverised, followed by solvent extraction to determine the physicochemical parameters. 200 ± 15mg of WBGP for different drying were weighed and extract was made using methanol/ethanol (as required) for further chemical analysis. Triplicate analyses were performed and the average values were recorded.

2.5.1 Chlorophyll Retention
Chlorophyll content was determined as reported by (Hiscox and Israelstam 1979)\(^{11}\), with minor modification. Powdered WBG was extracted using 95% ethanol. Using a UV-visible spectrophotometer, the filtered absorbance was determined
against a blank at 663 and 645 nm after reaction time of 4 hours. The sample content of chlorophyll a and chlorophyll b was determined by applying the following equations:

\[
\text{chlorophyll } a \left( \frac{\mu g}{g} \right) = (12.71A_{663} - 2.50A_{645}) \times \frac{\mu g}{g} \times 1000 \tag{4}
\]

\[
\text{chlorophyll } b \left( \frac{\mu g}{g} \right) = (22.88A_{645} - 4.67A_{663}) \times \frac{\mu g}{g} \times 1000 \tag{5}
\]

2.5.2 Determination of TPC and Antioxidant activity (DPPH)

WBGP was taken and extracted with 15ml ethanol, further made up to 100ml using ethanol and centrifuged for 10 minutes at 5000rpm and supernatant was collected. Gallic Acid preparation- 0.1g taken into 100ml distilled water. Then 10 ml taken from this and made up to 100ml using distilled water. The Folin-Ciocalteu procedure was followed as reported in (Ainsworth and Gillespie 2007) \[^1\]. Total phenols are expressed in terms of gallic acid equivalent (mg/g of dry mass):

\[
\text{Total phenolic content } \left( \frac{mg}{100g} \text{ gallic acid equivalent} \right) = \left( \frac{\text{absorbance of sample}}{\text{absorbance of standard}} \right) \times \left( \frac{\text{sample dilution weight}}{100} \right) \times \left( \frac{1 \text{ sample volume}}{100} \right) \tag{6}
\]

DPPH (1,1-diphenyl-2- picrylhydrazyl) method is used to determine the antioxidant activity of WBGP. In this method 1ml of DPPH solution and 10µl of 0.01g/ml of WBGP extract was diluted in methanol of volume 25ml (Wu and Ng 2008) \[^30\]. It is incubated at 25±20°C for 20 minutes by shaking the solution in a vortex. In spectrophotometer, at 517nm, the absorbance is noted, where methanol is used as blank control.

2.5.3 Ascorbic Acid (Harris and Ray 1935) \[^10\]

5 ml working standard was pipetted into a 100mL conical flask. 10ml 4% oxalic acid was added and titrated against the dye (V1 mL). End point was an appearance of pink color persisting for a few minutes. The amount of dye consumed is equivalent to the amount of ascorbic acid. 0.5g dried and powdered wild bitter gourd was extracted in 4% oxalic acid and made up to 100mL and centrifuged. 5mL of the supernatant was pipetted out and 10mL 4% oxalic acid was added and titrated against the dye (V2 mL).

\[
\text{Amount of ascorbic acid } \left( \frac{mg}{100g} \text{ sample} \right) = \frac{V_1}{V_2} \times \frac{100 mL}{100 mL} \tag{7}
\]

2.6 Statistical Analysis

The differences amongst Momordica Charantia var. muricata drying methods, VaCD, MW and FD against control i.e. Momordica Charantia var. muricata without drying operation samples were analysed using one-way ANOVA with Tukey’s test by SPSS software (ver. 20.0); p value < 0.05 was considered statistically significant.

3. Results and Discussion

3.1 Drying Characteristics (Drying time and Moisture content)

WBG had an initial moisture content of 87.18 ± 1.11%. In VaCD at 60°C, to reach final moisture content of 6.23 ± 0.25%, the drying process continued over prolonged period (48 hours). In MW at 360W output power, WBG took 10 minutes to reach 6.06 ± 0.18%. While in FD at -80°C, in order to achieve a final moisture content of 6.61 ± 0.13%, FD continued for a drying period of 24 hours. This was only after a brief 12 hour deep-freezing as a preliminary step to freeze drying. The moisture contents in % wet basis has been depicted in Table 1. These results show that MW at 360W for 10 minutes saves time to reach the 6% (w.b) final moisture content, and thus proved to be quicker in comparison to FD and VaCD. For MW drying of WBG, dewatering period was short. As a result of microwaves, an internal pressure generated in the sample, that lead to higher rate of energy transfer (Zahoor and Khan 2019) \[^31\]. The application of MW increased the temperature of water within the sample, which caused higher steam pressure and increased the drying rate. The order from least time consuming to most time consuming (for a similar final moisture content): MW< FD< VaCD. There was significant visible difference amongst different methods as represented in Fig 2.

3.2 Color and Water Activity

The total color value difference (ΔE) of dried WBG with FMC of around 6% (w.b) showed a high level of variance upon comparison amongst different drying methods (Table 2). The lowest value of 25.66 ± 5.16 being for VaCD, and a higher ΔE value of 30.69 ± 3.22 for MW drying, followed by FD with the highest ΔE value of 33.29 ± 0.91. The lightness index showed L* value highest in FD (56.50±1.58) followed by MW (52.22±2.17) and the least for VaCD (43.60±5.72), while the control (Fresh WBG) had L value of 24.09±1.21. The negative a* value depicts greenness, as the control had -6.77±0.17 value, the greenness decreased the least for FD, which retained a* value of -3.70±1.69, followed by MW (-2.14±0.55), while the VaCD recorded +7.91±0.16, which depicts the redness index and the difference noted is highest. The positive value of b* depicts yellowness index, and there was significant increase in the b* value. MW recorded 28.51±1.37 followed by VaCD 24.43±2.23 and least for FD at 23.99±1.12 (Fig 3). Least changes were noted in the FD sample in comparison to others.

The water activity of fresh WBG (untreated) sample was 0.97 with negligible deviation. The values are indicative of

![Fig 2: Dried and pulverized Wild bitter gourd powder (FMC 6% w.b)](http://www.chemijournal.com)
equilibrium relative humidity (ERH). MW treated WBG recorded highest \( a_w \) of 0.70 ± 0.02, while VaCD treated WBG showed \( a_w \) of 0.58 ± 0.01 and FD recorded the lowest \( a_w \) of 0.37 ± 0.03 (Table 1). These values were recorded corresponding to an average FMC of around 6% (w.b) of dried WBG. The lower \( a_w \) for FD implies better shelf life (Khalloufi, Giasson, and Ratti 2000) \(^{12}\). The effect of MW and VaCD drying conditions on \( a_w \) were found significant (\( p < 0.05 \)) in comparison to the control sample.

![COLOR VALUES](Fig 3: L* a* b* values of Fresh, VaCD, MW and FD samples using Hunter Color lab)

**3.3 Rehydration ratio**

Rehydration ratio stipulates the quality of the dried product. The rehydration curve for different drying methods are plotted (Fig. 4), in the graph – the x-axis denotes the time of dehydration i.e. 15, 30 and 45 minutes each, and the y-axis denotes the rehydration ratio. The variation in rehydration ratio was highest for shortest period of time i.e. 15 minutes with values of 4.14, 4.69 and 4.84 for FD, VaCD and MW, respectively. The variance amongst these drying techniques was noted at ± 0.36. The rehydration ratio at 45 minutes for 70 °C was highest for MW treated WBG with ratio of 7.28 indicating quality attribute allowing water to reenter through the pores (Okpala and Ekechi 2014) \(^{21}\).

![REHYDRATION CURVE](Fig 4: Variation in Rehydration ratio of WBG samples post-drying)

**3.4 Total phenolic content and Antioxidant activity**

The secondary metabolites play a significant role in body’s immune response against various pathological ailments. The total phenolic content was found to be 416.92 ± 9.20 GAE mg/100g for VaCD, the recorded data is in line with previous studies that suggested similar findings upon Vacuum drying (Stéger-Máté et al. 2011) \(^{26}\). The TPC for VaCD was highest due to liberation of phenolic content from the cellular matrix as a result of thermal treatment. In order of highest to lowest, the TPC were VaCD < MW (249.08 ± 10.12 mg GAE /100g) < FD (100.78 ± 1.84 mg GAE /100g). In, FD the sample is subjected to extended period exposure to lower temperature conditions and that is evident by the lower phenolic value while in MW as the heating takes place from the core and spreads outwards, it helps in release of phytochemicals and secondary metabolites at a rate better than FD, which is a result of microstructure changes effecting the extractability (Valadez-Carmona et al. 2017) \(^{27}\). The phenolic content helps in treatment of carbs absorption by inhibiting the absorption of enzymes that catalyzes the hydrolysis of starch.

The potential of antioxidant activity remains at helm of this study, with results showing close values amongst different techniques under subjected control conditions. The thermal exposure in VaCD at 60°C for prolonged periods impacted the antioxidant levels. The percentage inhibition was calculated using following equation:

\[
%\text{Inhibition} = \frac{(\text{Absorbance control} - \text{Absorbance sample})}{\text{Absorbance control}} \times 100
\]

The highest % inhibition was recorded by FD (74.61 ± 9.47) followed by MW (64.79 ± 7.22) and the least by VaCD (59.62 ± 0.19). The recorded data response demonstrates
effectiveness against oxidative stress. The analysis found that Freeze dried WBG was 74.61% effective in impeding DPPH.

3.5 Chlorophyll and Ascorbic content retention

The chlorophyll retention was highest in Freeze drying treatment (74.75%). The mean chlorophyll content in VaCD was recorded as 0.94 ± 0.01 mg/ml, while in MW it was recorded as 1.78 ± 0.02 mg/ml. The chlorophyll content is indicative result of environmental stress and nutrient availability in WBG. The higher drying temperature and time parameters proved to be most depreciable of chlorophyll content for VaCD and MW treated WBG. Ascorbic acid in control sample was recorded at 80.41 ± 1.73, which upon Vacuum-assisted convective drying showed enormous reduction (31.11 ± 0.95). The microwave treatment had a similar impact due to dipolar rotation of water molecules present in WBG and subsequent heat generation in short period, whereas the 24-hour low temperature drying under negative pressure proved to retain higher amount of ascorbic acid (49.06 ± 0.61). The water soluble vitamin was impacted by drying temperature, and subjected to oxidation (Marfil, Santos, and Telis 2008) [16]. The pretreatment had adverse impact upon the ascorbic acid content and showed higher rate of degradation upon drying. (Asami et al. 2003) [13].

Table 1: MCw.b, and water activity analysed against each drying method

| Treatments                        | Moisture content (w.b) % | a~  
|-----------------------------------|--------------------------|---  
| Microwave Drying                 | 6.06 ± 0.18              | 0.70 ± 0.02  
| Freeze Drying                    | 6.61 ± 0.13              | 0.37 ± 0.02  
| Vacuum-assisted Convectional Drying | 6.23 ± 0.25              | 0.58 ± 0.01  
| Control                           | 87.64 ± 1.11             | 0.97 ± 0.00  

Table 2: Selected drying methods and Colour value changes analysed

| Treatments                        | ΔE   | L       | a   | b   |
|-----------------------------------|------|---------|-----|-----|
| Microwave Drying                 | 30.69 ± 3.22| 52.22 ± 2.17| -2.14 ± 0.55| 28.51 ± 1.37 |
| Freeze Drying                    | 33.29 ± 0.91| 56.50 ± 1.58| -3.70 ± 1.69| 23.99 ± 1.12 |
| Vacuum-assisted Convectional Drying | 43.60 ± 5.72| 7.91 ± 0.16| 24.43 ± 2.23 |
| Control                           | -24.09 ± 1.21| -6.77 ± 0.17| 17.15 ± 0.39 |

Table 3: Change in Phenolic content and Retention of Chlorophyll content, Ascorbic acid upon selected drying method and Antioxidant Activity (Inhibition % - DPPH)

| Treatment      | TPC (mg GAE/100g) | Inhibition % (DPPH) | Chlorophyll content (mg/ml) | Ascorbic Acid(mg/100g) |
|----------------|-------------------|---------------------|-----------------------------|------------------------|
|                |                   |                     | R%                          | R%                     |
| Control        | 263.12 ± 35.68b   | 70.89 ± 5.63a       | 3.01 ± 0.03b               | 1.00 ± 0.02c           |
|                |                   |                     | 100                         | 100                    |
| MW             | 249.08 ± 10.13b   | 64.79 ± 7.22a       | 1.78 ± 0.02b               | 0.59 ± 0.01b           |
|                |                   |                     | 59.1                        | 39.25 ± 0.7b           |
|                |                   |                     | 48.81                       |                       |
| FD             | 100.78 ± 1.84b    | 74.61 ± 9.47a       | 2.25 ± 0.04b               | 0.75 ± 0.01c           |
|                |                   |                     | 74.6                        | 49.06 ± 0.61c           |
|                |                   |                     | 61.02                       |                       |
| VaCD           | 416.92 ± 9.20a    | 59.62 ± 0.19a       | 0.94 ± 0.01b               | 0.31 ± 0.00b           |
|                |                   |                     | 51.3                        | 31.11 ± 0.95a           |
|                |                   |                     | 38.69                       |                       |

(ΔR%) – percentage retention in comparison to control sample

4. Conclusion

Wild Bitter Gourd (Momordica charantia var. muricata), due to its higher concentration of secondary metabolites in comparison to Momordica charantia var. charantia was chosen for drying. The study aimed as to which drying process gives a better output in terms of intact nutrient composition, phytochemicals. The product was dried to a uniform final moisture content of 6% w.b alike using different drying methods, while MW took least, WBG was subjected to longer drying time in FD and VaCd. The lower water activity, which is considered essential for extended shelf life was noted in FD treated sample, while MW showed highest a~. The lightness index was reported highest in MW and FD treated WBG, while the color value ΔE was least for VaCd. FD treatment recorded least shrinkage percentage in comparison to Microwave dried WBG which showed highest shrinkage followed by VaCd. Rehydration was higher for MW dried WBG in comparison to FD, which is attributed to extended volume available in FD post sublimation of crystals from pores formed in freeze dried WBG. The phenolic content and antioxidant activity of VaCd is contradictory to those of other studies that reported Vacuum drying to have a higher inhibition (% DPPH) to Microwave drying. However, the present study was carried out using heated water as mode of convective heat transfer. Herein, it was accomplished using Titanium coils inside water duct insulated by Polyurethane. Thus, the contradicting results may be due to the difference in our experimental setup. Higher chlorophyll retention was noted in FD treated sample, while highest reduction recorded in VaCd, a similar result was accounted for Ascorbic acid retention. Heat treatment for prolonged period showed reduced ascorbic acid content in VaCd. The future prospects of synergistic effect of combined drying treatment of wild bitter gourd is a research area to be explored. The pretreatment of WBG also effects the phytochemical concentration and needs to be further inspected.

5. References

1. Ainsworth, Elizabeth A, Kelly Gillespie M. Estimation of Total Phenolic Content and Other Oxidation Substrates in Plant Tissues Using Folin–Ciocalteu Reagent. Nature Protocols 2007;2(4):875-77.
2. Aminah A, Anna PK. Influence of Ripening Stages on Physicochemical Characteristics and Antioxidant Properties of Bitter Gourd (Momordica charantia). International Food Research Journal 2011, 18(3).
3. Asami Denny K, Yun-Jeong Hong, Diane Barrett M, Alyson Mitchell E. Comparison of the Total Phenolic and Ascorbic Acid Content of Freeze-Dried and Air-Dried Marionberry, Strawberry, and Corn Grown Using Conventional, Organic, and Sustainable Agricultural Practices. Journal of Agricultural and Food Chemistry 2003;51(5):1237-41.
4. Bai Li-Yuan, Chang-Fang Chu, Po-Chen Chu, Wei-Yu Lin, Shih-Jiuan Chiu, Jing-Ru Weng. A Triterpenoid from Wild Bitter Gourd Inhibits Breast Cancer Cells. Scientific Reports 2016;6(1):1-10.
5. Cariño Ina, Bitter Melon. New England Review 2019;40(3):102-3. https://doi.org/10.1353/ner.2019.0094.
6. Chakravarty HL. Cucurbits of India and Their Role in the Development of Vegetable Crops. Biology and Utilization of the Cucurbitaceae (DM Bates, RW Robinson, and C. Jeffrey, Eds.). Cornell University Press, Ithaca, NY 1990, 325-34.

7. FAO. The 74th Session of the United Nations General Assembly 2019. http://www.fao.org/new-york/news/detail/en/c/1256329/#:~:text=The%2074th%20Session%20of,%20Year%20of%20Fruits%20and%20Vegetables. & text = The resolution is the latest, support agriculture and healthy nutrition.

8. Ghosh, Iphieta, Biplab Kumar Bhowmick, Sumita Jha. Cyto genetics of Two Indian Varieties of Momordica charantia L. (Bittergourd). Scientia Horticulturae 240 (July 2017), 333-43. https://doi.org/10.1016/j.scienta.2018.06.027.

9. Harini R, Tejas Muthal DV, Chidanand, Sunil CK. Study on Physiochemical Quality Characteristics of Tomato Slices Dried Using Innovative Drying System. IJCS 2019;7(3):4719-23.

10. Harris LJ, Ray SN. Determination of Plasma Ascorbic Acid by 2, 6-Dichlorophenol Indophenol Titration. Lancet 1935;1(7):1.

11. Hiscox JD, Israeilstam GF. A Method for the Extraction of Chlorophyll from Leaf Tissue without Maceration. Canadian Journal of Botany 1979;57(12):1332-34.

12. Holman, Naomi, Peter Knighton, Partha Kar, Jackie O’Keefe, Matt Curley et al. Risk Factors for COVID-19-Related Mortality in People with Type 1 and Type 2 Diabetes in England: A Population-Based Cohort Study. The Lancet Diabetes & Endocrinology 2020;8587(20):1-11. https://doi.org/10.1016/s2213-8587(20)30271-0.

13. Huang, Ting-ni, Kan-Ni Lu, Yi-Ping Pai, Chin Hsu, Ching-jang Huang. Role of GLP-1 in the Hypoglycemic Effects of Wild Bitter Gourd. Evidence-Based Complementary and Alternative Medicine 2013.

14. Khalloufi S, Giasson J, Ratti C. Water Activity of Freeze Dried Mushrooms and Berries. Canadian Agricultural Engineering 2000;42(1):51-56.

15. Krokida MK, Karathanos VT, Maroulis ZB. Effect of Freeze-Drying Conditions on Shrinkage and Porosity of Dehydrated Agricultural Products. Journal of Food Engineering 1998;35(4):369-80.

16. Marfil PHM, Santos EM, Telis VRN. Ascorbic Acid Degradation Kinetics in Tomatoes at Different Drying Conditions. LWT-Food Science and Technology 2008;41(9):1642-47.

17. Marques, Luanda G, Ana Silveira M, José T Freire. Freeze-Drying Characteristics of Tropical Fruits. Drying Technology 2006;24(4):457-63.

18. Meeteren U, Van H, Van Gelder, Van Ieperen W. Reconsideration of the Use of Deionized Water as Vase Water in Postharvest Experiments on Cut Flowers. Postharvest Biology and Technology 1999. https://doi.org/10.1016/S0925-5214(99)00050-2.

19. Mohammed Majeed, Lynda D Wickham. Extension of Bitter Gourd (Momordica charantia L.) Storage Life through the Use of Reduced Temperature and Polyethylene Wraps. Journal of Food Quality 1993;16(5):371-82.

20. Mujumdar, Arun S, Chung Lim Law. Drying Technology: Trends and Applications in Postharvest Processing. Food and Bioprocess Technology 2010;3(6):843-52.

21. Okpala, Laura C, Constance Ekechi A. Rehydration Characteristics of Dehydrated West African Pepper (Piper Guineense) Leaves. Food Science & Nutrition 2014;2(6):664-68.

22. Paul, Ananya, Ss Raychaudhuri. Medicinal Uses and Molecular Identification of Two Momordica Charantia Varieties-a Review. EJ Bio 2010;6(2):43-51. http://www.ejbio.com/pps/2010/43/pdf?origin=publication_detail.

23. Rai M, Pandey S, Kumar S. Cucurbit Research in India: A Retrospect. IXth EUCARPIA Meeting on Genetics and Breeding of Cucurbitaceae 2008.

24. Savoia, Dianella. Plant-Derived Antimicrobial Compounds: Alternatives to Antibiotics. Future Microbiology 2012;7(8):979-90.

25. Singh, Uadal, Sagar VR. Effect of Drying Methods on Nutritional Composition of Dehydrated Bitter Gourd (Momordica charantia L.) Rings. Agriculture for Sustainable Development 2013;1(1):83-86.

26. Stéger-Maté, Mónika, Beatrix Nótin, Réka Juhász, Balázs Verasztó, Dávid Jakab et al. Effect of Vacuum Drying on Blackcurrant’s Antioxidant Components. In International Congress on Engineering and Food 2011, 22-26.

27. Valadez-Carmona, Lourdes, Carla Patricia Plazola-Jacinto, Marcela Hernández-Ortega, Maria Dolores Hernández-Navarro, Francisco Villarreal et al. Effects of Microwave, Hot Air and Freeze-Drying on the Phenolic Compounds, Antioxidant Capacity, Enzyme Activity and Microstructure of Cacao Pod Husks (Theobroma cacao L.). Innovative Food Science & Emerging Technologies 2017;41:378-86.

28. Wang, Hai Ou, Qing Quan Fu, Shou Jiang Chen, Zhi Chao Hu, Huan Xiong Xie. Effect of Hot-Water Blanching Pretreatment on Drying Characteristics and Product Qualities for the Novel Integrated Freeze-Drying of Apple Slices. Journal of Food Quality 2018. https://doi.org/10.1155/2018/1347513.

29. Weinstein, Aviv M, Paola Rosca, Liana Fattore. Innovative Food Science & Emerging Technologies 2012;7(8):979-90.

30. Wu Shu Jing, Lean Teik Ng. Designer Drugs Pose a Major Risk for Public Health. Frontiers in Psychiatry 2017;8:156.

31. Wu Shu Jing, Lean Teik Ng. Antioxidant and Free Radical Scavenging Activities of Wild Bitter Melon (Momordica charantia Linn. Var. Abbreviata Ser.) In Taiwan. LWT - Food Science and Technology 2008;41(2):323-30. https://doi.org/10.1016/j.lwt.2007.03.003.

32. Zahoor, Insha, Mohammad Ali Khan. Microwave Assisted Convective Drying of Bitter Gourd: Drying Kinetics and Effect on Ascorbic Acid, Total Phenolics and Antioxidant Activity. Journal of Food Measurement and Characterization 2019;13(3):2481-90.

33. Zhang, Min, Juming Tang, Mujumdar AS, Wang S. Trends in Microwave-Related Drying of Fruits and Vegetables. Trends in Food Science & Technology 2006;17(10):524-34.