RESEARCH ARTICLE

Bioactive Compounds, Vitamins and Minerals Composition of Freeze-dried *Grewia asiatica* L. (Phalsa) Pulp and Seed Powder

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Abstract

Present study was directed to analyze and compare the bioactive compounds (total phenols, total anthocyanins), vitamins (ascorbic acid, β-carotene, vitamin A), minerals including Ca, Mg, Na, P, K, Fe, Cu, Zn, Co, Mn and heavy metals (Cd, Hg, Pb) of freeze-dried (lyophilized) phalsa pulp and seed powder. In lyophilized pulp powder (LPP) higher amount of total phenols (78.11 mg/100g), total anthocyanin (82.94 mg/100g), ascorbic acid (5.21 mg/100g), β-carotene (0.54 µg/100g), vitamin A (0.89 I.U.) were observed than lyophilized seed powder (LSP). Na, K, Mg and Co (0.41, 0.39, 1.08, 0.46 mg/100g, respectively) were higher in LPP as compared to LSP (0.29, 0.11, 0.76 and 0.40 mg/100g, respectively) whereas, Ca, P and Cu were detected more in LSP. This study opens the prospect of using dry phalsa powder in the preparation of various nutraceutical and functional foods for their therapeutic as well as prophylactic purposes.

Keywords: Bioactive compounds, Lyophilization, Minerals, pulp powder, Seed powder, Vitamins

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Introduction

Nowadays, bioactive compounds, are establishing importance in nutritional as well in the medicinal field owing to their immense potential of treating various health issues. Raised interest in deep-colored fruits and phytochemicals have been observing recently, has contributed to increased consumption of underutilized fruits as a potential source of bioactive compounds. *Grewia asiatica* L. is one of the most popular underutilized, minor fruit crops of Indian origin Kacha *et al.* (2014). Phalsa plant is a small shrub belonging to the family *Tiliaceae*, with the genus *Grewia,* comprises about more than 140 species. It is widely distributed in India, South Africa, Pakistan, Southeast Asia, and USA Sinha *et al.* (2015). In India, it is commercially grown in Punjab, Haryana, Rajasthan, Uttar Pradesh, Madhya Pradesh, and is also cultivated on a limited scale in the states of Gujarat, Bihar, Tami Nadu, Maharashtra and West Bengal Kumar *et al.* (2014). The plant yields delicious fruits of edible quality and can withstand drought and grown under adverse climatic conditions, Debnath *et al.* (2011). Mainly it is utilized as fresh fruit as well as a refreshing drink during hot summer and medicinal resources for treating various ailments including heart and liver disorders, cancer, anorexia, hiccough, asthma, stomatitis, diarrhea, throat infection, tuberculosis, cataract and Parkinson's disease, etc. Srivastva *et al.* (2012). Phalsa fruit is abundant in several bioactive compounds like anthocyanins, phenolics, flavonoids, tannins, and antioxidant vitamins Tiwari *et al.* (2014). It also needs to be mentioned here that ripe fruits contain 50–60% juice, 10–11% sugar, and 2.0-2.5% acid with a good quantity of vitamin A as well as C and a fair source of phosphorus and iron Kacha *et al.* (2012).

Furthermore, phalsa seeds are also nutritionally rich and eaten along with fruits. However, detailed studies regarding compositional aspects of dry pulp and seed powder are yet not fully known and require further analysis. In India, this fruit has not yet gained much popularity amongst fruit growers and processed fruit industries, in spite of its large nutritional and medicinal attributes. This fruit is still growing unattended; not following any systematic approach, as knowledge and regular cultivation of this fruit are still scanty.

On the other hand, it is very less studied fruit hence little utilized by the food and pharmaceutical industries. Conversion of fresh phalsa into dry powder is a very effective method for preservation of fruit, as it results in extension of shelf life, throughout year availability, and use of fruit for various health-promoting nutraceutical and functional foods products. There are many advantages of lyophilization over conventional drying like minimum chemical decomposition, water removal without too much heating, and more stability of the dried product. Therefore, it was necessary to strengthen the research about freeze-dried phalsa powder to promote its industrialization, and this study will also provide useful scientific data for documentation as well as for further studies.

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Materials and methods

Materials

Fresh phalsa fruits of variety Sharbati were harvested at the Central Fruit Farm, Hisar (Haryana), at their 'ready-to-eat' ripening stage during session 2017-18. The present research work was carried out in the laboratories of the Department of Food Technology, Guru Jambheshwar University of Science and Technology (Hisar), Haryana. After thorough washing using tap water, round-shaped, big-sized, and uniform dark purple-colored fruits were selected for preparation of dried powder. Pulp and seeds (separated using stainless steel knife) were dried using freeze-drier (CHRIST- Alpha 2/4 LD plus, Germany) separately. Homogeneous dried powders were prepared by grinding the dried pulp and seed material using an electric mixer grinder (Sujata). Powder samples were packed in LDPE zip-lock pouches and kept in refrigeration at -5±2°C until further analysis. Figure 1 represents the lyophilized pulp (A) and seed (B) powder. All the chemicals used were of analytical grade and purchased from Hi Media Laboratories Pvt. Ltd., Sigma Aldrich and CDH, Merck Pvt. Ltd.

Methods

Total phenol

Total phenol content of lyophilized pulp powder (LPP) and lyophilized seed powder (LSP) was assessed by the Folin-Ciocalteau (FC) method as described previously by Anesini *et al.* (2008). For extraction of phenolic compounds, 2 g powder sample was extracted with 400 ml distilled water in boiling water bath for 30 minutes. For estimation, FC reagent (1:10 dilute with distilled water) 0.5 ml was added to the test tube containing 1 mL of the extract. After 5 minutes, 1 mL saturated sodium carbonate (Na_2CO_3) was added to the test tube, and 10 mL final volume was made with distilled water. Then the absorbance was measured spectrophotometrically at 765 nm after 30 min and expressed in terms of gallic acid equivalent (GAE). The standard curve was prepared using 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 mL of standard gallic acid solution.

Total anthocyanin

The total anthocyanin content of LPP was determined using Srivastava and Kumar (2002) method. Five g powder sample was macerated with 10 mL ethanol-acid solution in pestle motor. Then the solution was transferred in brown glass bottle and was kept in the refrigerator (5 ± 1^0C) overnight. After filtration, absorbance was measured at 535 nm using UV-vis Spectrophotometer.

Ascorbic acid

Ascorbic acid content was obtained by following the Association of Official Agricultural Chemists (AOAC) (2005) protocol, which involves the L-ascorbic acid-induced color reduction of 2, 6-dichlorophenolindophenol solution to a colorless substance. Results were represented in g/ 100g.

β-carotene and vitamin A

β-carotene of the samples was estimated by following the method described by Srivastava and Kumar (2002). Two g dry powder samples of each (pulp and seed) were taken and macerated with 15 ml acetone in a pestle mortar. Then 15 ml petroleum ether was added to the separating funnel, and two layers were formed. The lower layer was discarded, and the upper layer was collected in 100 mL volumetric flask. Final volume 100 ml was prepared with petroleum ether, and optical density was measured at 452 nm using petroleum ether as blank. Vitamin A was calculated using the following formula.

$$\beta\text{-carotene } (\mu g/\ 100g) = \frac{O.D. \times 13.9 \times 10^4 \times 100}{Wt.\ of\ sample \times 560 \times 1000}$$

$$\text{Vitamin A (I. U.)} = \frac{\beta\text{- Carotene } (\mu g/\ 100g)}{0.6}$$

Determination of minerals and heavy metals

For the estimation of minerals and heavy metals, dry ash was prepared from LPP and LSP by adopting standard protocols as outlined in AOAC (2005). To the crucible containing white ash,

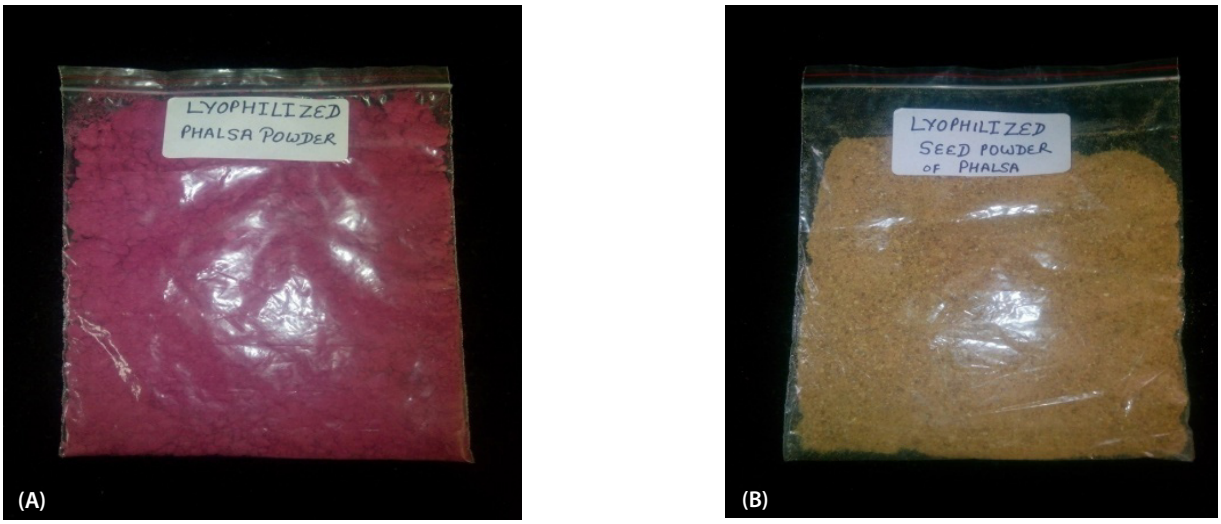

Figure 1(A-B): Freeze-dried phalsa pulp powder (A) and freeze-dried phalsa seed powder (B)

20 mL HCl (1:1), and 1 mL of conc. HNO_3 were subsequently added, followed by evaporated to dryness. After adding 4 ml of HCl solution, the contents of the crucible were warmed for a few minutes over a boiling water bath. After filtration, 100 mL final volume was made with distilled water.

Mineral elements and some heavy metals, including calcium, copper, iron, manganese, magnesium, zinc, lead, cobalt, cadmium, and mercury, were determined using an atomic absorption spectrophotometer [AAS (GBC 932 plus, Advance Scientific, Australia)]. A series of graded concentrations were prepared from the standard chemicals (AR grade) of the mineral mentioned above elements. Calibration of AAS was carried out with a respective standard solution of each mineral in between the estimation of the sample for obtaining the calibration values constant during the running of the samples. For each of the above-mentioned minerals, calibration curves were plotted between absorption and standard concentration (µg/ml).

Mineral elements viz., sodium, and potassium were determined using a flame photometer (Elico, CL-378). Phosphorus was estimated colorimetrically, following the method described by Chapman and Pratt (1982). From the standard phosphate solution, a series of graded concentrations (0.2–2 mL) was prepared, and to each 50 mL of standard phosphate solution, a 2 mL ammonium molybdate solution was added. Two drops of stannous chloride were then added, and the appearance of blue color indicated the presence of phosphorus. Absorbance was measured at 690 nm, and a standard curve was prepared between absorbance and phosphorus concentration. Above mentioned same procedure was followed for the samples.

Statistical analysis

To verify the statistical significance means ± standard deviations of three independent measurements were determined. One-way ANOVA was performed using SPSS 15 software to determine the significance of differences between analytical results at $p < 0.05$ significance level. Means were compared by Duncan (Duncan's multiple range tests).

Results and discussions

Bioactive compounds

Freeze-dried powders (LPP and LSP) of phalsa were determined for bioactive compounds (total phenols, total anthocyanin) and vitamins (Ascorbic acid, β–carotene, Vitamin A) (Table 1). A significant variation ($P < 0.05$) was observed in the amount of bioactive compounds and vitamins between LPP and LSP. It was noted that LPP had 78.11 mg/100g total phenols, whereas, in LSP 23.90 mg/100g total phenols were observed. Comparatively less amount of total phenols were observed in LSP than LPP. These observed differences might be because fruit pulp and seeds are separate botanical parts of the fruit and exhibit different phytochemicals composition. To the best of our knowledge, no analysis has been performed for comparing the bioactive compounds, vitamins, and minerals contents of freeze-dried phalsa pulp and seed powder.

On the other hand, various reports have been presented in literature about the influence of freeze-drying on bioactive compounds and vitamins for other fruits. The amount of total phenols in fresh sweet cherry cultivars was reported to be varied from 58.31 to 115.41 mg GAE/100 g (fresh weight basis) Hayaloglu and Demir (2015). Total phenols of LPP were found lies in between the range observed for cherry cultivars, while LSP showed lower values. Sofia *et al.* (2014) studied two types of mulberry (white and black) for polyphenols content and reported that black mulberry possessed the highest total phenols than white mulberry fruits. Thus, it can be considered that dark-colored fruits are good sources of phenolics. The observed differences in total phenol content of LPP and LSP might be the color attribute of both powders.

Total anthocyanin content was 82.94 mg/100g in LPP whereas, this pigment was not determined in LSP. Pangotra (2016) observed 74.12 mg/100 g anthocyanin content in fresh phalsa fruit. The obtained amount of anthocyanins in LPP was more as compared to fresh phalsa fruit, which attributed to rise in the dry matter content owing to the evaporation of water. Crude methanolic extracts of this fruit showed total phenols 144.11 mg GAE/g and total anthocyanin 4.88 mg/kg Srivastava *et al.* (2012). Kaur and Kapoor (2005) also obtained moderate amounts of total phenols i.e., 55-87 mg/100g in phalsa. The importance of anthocyanins and phenols in food goes beyond their role as natural pigments. Phalsa is a dark-colored fruit, and this dark purple color of phalsa is attributed to the presence of anthocyanins Singh *et al.* (2009). Hot air drying of materials causes degradation of important flavors and bioactive compounds, as reported by Stepien *et al.* (2019). A higher concentration of bioactive compounds in freeze-dried phalsa as compared to fresh could be attributed to the greater extraction efficiency. Freeze drying was also proved the best method for maintaining the amount of phenolics and anthocyanins in dried grapes Coklar and Akbulut (2017).

Table 1: Bioactive compounds and vitamins content and of lyophilized pulp powder (LPP) and lyophilized seed powder (LSP) of phalsa

	Bioactive compounds (FW)		*Vitamins (FW)*		
Sample	*Total phenols (mg GAE/100g)*	*Total anthocyanin (mg/100g)*	*Ascorbic acid (mg/100g)*	*β–carotene (µg/100g)*	*Vitamin A (I.U.)*
LPP	$78.11^{a} \pm 0.13$	$82.94^{a} \pm 0.44$	$5.21^{a} \pm 032$	$0.54^{a} \pm 0.14$	$0.89^{a} \pm 0.24$
LSP	$23.90^{b} \pm 0.28$	-	$3.75^{b} \pm 0.43$	$0.33^{b} \pm 0.05$	$0.54^{b} \pm 0.08$

Values are mean ± SD of three independent determinations. Where, LPP- Lyophilized pulp powder and LSP-Lyophilized seed powder. Mean values with different superscript within a column are significantly different. FW- fresh weight basiss

Freeze-drying method produces dried products with greater porosity (80%–90%) as compared to convective-, microwave- and vacuum-drying methods Joardder *et al*. (2015).

Vitamins

Greater amount of ascorbic acid (5.21mg/100g), β-carotene (0.54 µg/100g), vitamin A (0.89 I.U.) were observed in LPP as compared to LSP (3.75 mg/100g ascorbic acid, 0.34 µg/100g β-carotene, 0.54 I.U. vitamin A). Pangotra (2016) reported that vitamin C varied from 4.38 to 32.10 mg/100g in fresh phalsa fruit. Freeze-dried powder of *Grewia sapida* also showed a similar amount of vitamin C Islary *et al.* (2016) as LPP of phalsa showed. LPP of phalsa was found to have a lower value (7.50 mg/100g) of ascorbic acid content as compared to fresh fruits (5.21 mg/100g). The similar differences were also recorded by Mishra *et al.* (2009) for the Chakiya variety of Amla. In their finding, fresh fruit was found to have more ascorbic acid content as compared to lyophilized powder. It might be due to the processing of fresh fruits into dried powder. When pulp and seeds were separated from each other with the help of a knife and dried using freeze drier, the oxidation process took place. This may be the reason for low ascorbic acid content in LPP. Owing to differences in extraction processes, analysis methods, non-availability of literature, and way of results expression (wet or dry weight basis), comparison of our results with existing studies become complicated.

Minerals and heavy metals

Minerals and heavy metals detected in LPP and LSP of phalsa and their amounts (mg/100g FW) are represented in Table 2. A significant variation in mineral composition was observed between LPP and LSP. LPP showed Na, K, Mg and Co (0.41, 0.39, 1.08, 0.46 mg/100g, respectively) in higher amount as compared to LSP (0.29, 0.11, 0.76 and 0.40 mg/100g, respectively). Ca, P, and Cu were found higher in LSP than LPP, whereas Fe was found in almost equal amount (non-significant difference). Range of heavy metals (lead and cadmium) in LPP and LSP did not exceed the maximum permissible limit and relatively low as compared to the fruits and vegetables collected from Kuwait Husain *et al.* (1995) and also from strawberries, cherries, black current, pears and apples as studied by Krejpcio *et al.* (2005). Mercury was not detected in all the three samples analyzed. It is not possible to compare the values which vary from study to study for the phalsa minerals as many factors may influence the mineral composition of fruits. However, it is important to mention that phalsa fruit and seed are the good sources of macro and micronutrients. Heavy metals including lead (Pb) and cadmium (Cd) are dangerous to human health because Pb causes serious effects to all age people (especially young age people) as well as animals and Cd is a very toxic and carcinogenic element Krejpcio *et al.* (2005).

Conclusion

The seasonality and short period availability of phalsa fruit limit the throughout year consumption of it, so new products

Table 2: Mineral profile and heavy metals content of lyophilized pulp power (LPP) and lyophilized seed powder (LSP) of phalsa

	Minerals (FW)								*Heavy metals (FW)*			
Sample	*Na (mg/100g)*	*K (mg/100g)*	*Ca (mg/100g)*	*P (mg/100g)*	*Fe (µg/100g)*	*Mg (mg/100g)*	*Co (mg/100g)*	*Cu (µg/100g)*	*Zn (µg/100g)*	*Mn (µg/100g)*	*Cd (µg/100g)*	*Hg (mg/100g)*	*Pb (mg/100g)*
LPP	$0.41^{a} \pm 0.42$	$0.39^{a} \pm 0.21$	$1.51^{b} \pm 1.10$	$36.10^{b} \pm 0.02$	$28.80^{b} \pm 0.07$	$1.08^{a} \pm 0.98$	$0.46^{a} \pm 1.0$	$72.70^{b} \pm 0.10$	$1.28^{a} \pm 0.14$	$12.12^{a} \pm 1.07$	$0.02^{a} \pm 0.21$	ND	$0.01^{a} \pm 0.01$
LSP	$0.29^{b} \pm 0.04$	$0.11^{b} \pm 0.05$	$2.02^{a} \pm 0.08$	$43.13^{a} \pm 0.05$	$29.48^{b} \pm 0.82$	$0.76^{b} \pm 1.01$	$0.40^{b} \pm 0.12$	$0.86^{a} \pm 0.02$	$0.39^{b} \pm 0.53$	$1.51^{b} \pm 1.10$	ND	ND	$0.01^{a} \pm 1.02$

Values are mean±SD of three independent determinations. Where, ND– Not detected, LPP- Lyophilized pulp powder and LSP- Lyophilized seed powder. Mean values with different superscript within a column are significantly different. FW- fresh weight basis

in the form of dry powder may offer a very good alternative to fresh consumption and utilization as high-quality natural food ingredients for the preparation of various health-boosting food formulations with very attractive sensory attributes. It is clear from the above study that phalsa, along with seed in dried form, is a promising indigenous fruit in terms of bioactive compounds and nutritional aspects. Bioactive compounds are credited to the antioxidant properties of phalsa. Phyto-constituents present in phalsa can be isolated, characterized, and screened for various activities. Considering the amount of nutritional as well as bioactive compounds observed in lyophilized pulp and seed powder, it can be concluded that dried phalsa fruit is an important source of these constituents and it deserve to be studied and utilized as further for beneficial effect in human health.

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