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Influence of Drying Method on Total Flavonoid Content of Phalsa (*Grewia asiatica*) Fruit

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Abstract- This study aimed to assess the effects of thermal drying (tray drying) and non-thermal drying (freeze drying) on the total flavonoid content (TFC) of tall and dwarf cultivars of phalsa (Grewia asiatica) fruit. Before analysis, ethyl alcohol extract of each cultivar was screened for the presence of flavonoids. Appearance of yellow coloured precipitates in dwarf cultivar and greenish yellow coloured precipitates in tall cultivar, confirmed the presence of flavonoids. Results of this study revealed that freeze dried powder of dwarf cultivar contain highest amount of TFC (0.437 ± 0.02 mg QE/ml) followed by freeze dried tall cultivar (0.396 ± 0.04 mg QE/ml) as compared to tray dried tall (0.235 ± 0.01 mg QE/ml) and dwarf (0.349 ± 0.04 mg QE/ml) cultivars. In conclusion, freeze drying was found to be a more effective method for maintaining TFC and dwarf cultivar has greater amount of total flavonoids compared to tall cultivar.
Keywords- Phalsa cultivars, Tray drying, Freeze drying, Total flavonoids, Quercetin

I. INTRODUCTION

Being a basic and central part of our food chain (Haq, et al. 2015), fruits and vegetables are greatly valued for their nutritional as well as curative effect against various degenerative diseases like cardiovascular, cancer, cataract, diabetes, and neurodegenerative diseases i.e. Alzheimer's and Parkinson's owing to the presence of various antioxidants (Loganayaki and Manian, 2010).

Phalsa (*Grewia asiatica*) having huge nutritional and medicinal significance is counted among the underutilized minor fruit crops of India (Haq, et al. 2013).The genus *Grewia* belongs to the family Tiliaceae comprising approximately 150 species among which nearly 40 species are found in India and phalsa is one of them having several bioactive compounds like anthocyanins, phenolics, flavonoids, tannins and vitamins which exhibit very high antioxidant activity (Tiwari, et al. 2014). This small size berry like fruit is native to the Indian subcontinent and Southeast Asia (Maury, et al. 2012) and because of perishable nature; transport is difficult, so it is not available throughout the country (Dave, et al. 2015). Ripe fruits are subacidic, exert cooling effect and are used for making excellent juice, squash, jams, pies and chutneys as well as consumed as table fruit by the people of all age group during hot summers (Kumar, et al. 2014). The fruit is considered to have astringent and stomachic properties and possess anti malaria and anti ulcer effects (Srivastava, et al. 2012). Triterpenoids, fatty component, flavonoids (quercetin, quercetin-3-O-β-D-glucoside and naringenin-7-O-β-D-glucoside), steroids, saponins and tannins are the main phytochemicals present in this fruit and eye-catching crimson red to dark purple colour of the fruit is because of anthocyanin pigment (Tiwari, et al. 2014). Unripe phalsa fruit alleviates inflammation and it may help in fever reduction, curing respiratory, cardiac, blood disorders (Paviaya, et al. 2013), heat troubles and constipation (Abid, et al. 2012).

Flavonoid content of foods is a topic of interest since the early 1980s (Harnly, et al. 2006) and received a considerable attention at present owing to their antioxidant, antimutagenic and antitumor activities (Rohman, et al. 2010). These are the common dietary bioactive compounds present in fruits, vegetables and cereals in small quantity (Prasad, et al. 2012) and are affected by growth, season, geographical location, climate, weather, soil type, degree of ripeness, processing, storage and other conditions (Islek, et al. 2014). Moreover, flavonoids also provide attractive bright shades of blue, scarlet and orange, in leaves flowers and fruits (Pietta, 2000). Tropical fruits like avocado, phalsa, jamun, etc. that are underutilized at present, possess immense potential for satisfying the demand of society with changing food habits for nutritious and healthy natural foods (Dave, et al. 2015). Recently, there have been great efforts to find safe and potent natural antioxidants from various plant sources (Rohman, et al. 2010). For this reason, both composition and content of flavonoids in foods has been the subject of much research today. Several underutilized fruits not received much consideration as antioxidant sources and this could be due to lack of information on nutritional compositions. Hence, in spite of being highly versatile, this fruit is still underutilized. Considering the above scenario, this study was designed to find out the effect of drying methods (thermal and non-thermal) on total flavonoids content of phalsa cultivars.

II. MATERIALS AND METHODS

A. Chemicals

Aluminum chloride, sodium nitrite, sodium hydroxide, ethyl alcohol, lead acetate, ethanol and quercetin used for this study were of analytical grade. Standards and other chemicals were prepared using double distilled water.

B. Sample collection

Phalsa fruits of tall and dwarf cultivars of variety Sharbati, analysed in this study were procured from Central Fruit Farm Hisar (Haryana), India in the month of June and July 2015. These were picked in their proper maturity stage during early hours of the day and were transported in ice boxes from farm to laboratory due to highly perishable nature. Immediately after reaching in laboratory (Fruits and Vegetable Processing laboratory), fruits were thoroughly washed with running tap water and shorted for uniform size, colour and free from any physical damage or visible signs of disease occurrence. Selected phalsa were subjected to drying in open air for removal of water droplets from outer surface and further analysis was carried out as follows.

C. Sample preparation

1) *Tray drying method:* Fruits of both the cultivars were dried in tray drier (Oven 300, NSW 354) at 45°C for 3 days. Dried samples were grinded using commercial grinder and were stored in air-tight glass containers separately at 4°C for further analysis.
2) *Freeze drying method:* Fruits of both the cultivars were placed in ultra low temperature freezer at – 80 °C (U410) for 12 hours in petriplates separately before freeze drying. A vertical freezer, designed and built by Germany (CHRIST- Alpha 2/4 LD plus Germany) was used to freeze-dry the samples. Both the samples were subjected to freeze drying for 24 hours at -75°C. Then each sample was grinded using commercial grinder and stored in air-tight glass containers at 4°C individually, until further analysis.

D. Flavonoids screening

Duplicate representative samples of each (freeze-dried and tray dried) powder of both tall and dwarf cultivars were taken for the analysis. Two gram powder of each sample was separately added to the 100 ml ethyl alcohol followed by extraction using rotary shaker (NSW-200) for 8-10 hours. After filtration, crude ethanolic extracts of both the cultivars were subjected to preliminary qualitative screening to confirm the presence or absence of plant secondary metabolite i.e. flavonoids, following the standard test for flavonoids (Bhandary, et al. 2012). Twenty ml lead acetate (10 percent) was added to 5 ml aliquot of crude extracts of each sample and after 1-2 minutes results were observed.

E. Preparation of extract

Extraction is the first and most important step for determination of flavonoids. Flavonoids from dried powder of each cultivar were extracted by following the method of (Zhishen, et al. 1999). One gram powdered sample of each powder was added to 100 ml ethanol individually and Soxhlet extraction was carried out at 60 °C for one hour. Extractions under the same conditions were repeated three times for each sample. After filtration, combined crude extracts of each sample were separately stored in amber colored glass bottles at -5 °C for prevention from oxidative damage until further analysis.

F. Determination of total flavonoids content (TFC)

Total flavonoids content of each sample extract was determined using aluminium chloride colorimetric assay described by (Prasad, et al. 2012) with few modifications. In order to investigate total flavonoids, an aliquot (2 ml) from each extract was poured in 5 ml volumetric flasks separately and 0.2 ml, 5% $NaNo_2$ was added. After 5 minutes, 0.2 ml (10% $Alcl_3$) was added and mixed properly. After 6 minutes, 2 ml (1M) sodium hydroxide was added and final volume was made 5 ml with distilled water. After 15 minutes, absorbance was measured at 415 nm using UV spectrophotometer (G10S UV-VIS). Since the quercetin content is high in phalsa fruit, it was used as a standard for the calibration curve.The samples were prepared in triplicates for each analysis and the mean value of absorbance was obtained. A blank containing all reagents except samples of extracts was prepared. Stock solution of 100 ppm was prepared and from that 0.0, 0.4, 0.8, 1.2, 1.6, 2.0 ml aliquots were taken for calibration curve construction. Based on the measured absorbance, the concentration of flavonoids was read (mg/ml) on the calibration line; then, the content of flavonoids in the extracts was expressed in terms of quercetin equivalent (mg of QE/ ml of extract).

G. *Statistical Analysis*

All experimental measurements were conducted in triplicates and results obtained were expressed as mean ± standard deviation.

III. RESULTS AND DISCUSSIONS

A. Screening for flavonoids

Flavonoids, possessing nutritional and therapeutic value are a group of ubiquitous polyphenolic compounds and use of these natural antioxidant in food and other biological materials is presumed safe (Tamuly, et al. 2013). Appearance of yellow precipitates in dwarf cultivar (tray dried and freeze dried) and yellow - greenish precipitates in tall cultivar (tray dried and freeze dried) confirmed the presence of flavonoids as depicted in Table 1. In vivo and in vitro qualitative phytochemical screening of *Grewia* species by (Sharma and Patni, 2013) confirmed the presence of different phytoconstituents in seven different solvents extract of leaf, stem and callus. Parul, et al. (2012) also reported that alcoholic extract of dried fruit powder of *Grewia asiatica* contains flavonoids.

TABLE 1: PRELIMINARY SCREENING OF PHALSA CULTIVARS FOR FLAVONOIDS

Phalsa cultivars	Lyophilized powder			Tray dried powder		
	Test applied	Observations	Results	Test applied	Observations	Results
Tall	Lead acetate test	Greenish yellow colored ppt	+	Lead acetate test	Yellow colored ppt	+
Dwarf	Lead acetate test	Greenish yellow colored ppt	+	Lead acetate test	Yellow colored ppt	+

B. Influence of drying process on total flavonoid content

To perform the calculations of total flavonoid content in the samples, a standard curve is needed which is obtained from a series of different quercetin concentrations. The calibration curve of quercetin to determine flavonoid content is shown in Fig. 1. Total flavonoid content of ethanol extract of all the samples is compiled in Table 2. The results shows that freeze dried sample of dwarf cultivar has the highest TFC followed by the freeze dried sample of tall cultivar as compared to tray dried samples. Among tray dried samples dwarf cultivar shows TFC more than tall cultivar as depicted by Fig. 2. Similar results were also reported by (Periche, et al. 2015) for *Stevia rebaudiana* leaves and by (Irondi, et al. 2013) for Carica papaya seeds. They recommended that freeze drying is the most suitable method of drying if an extract with sufficient antioxidant properties, maximum phytochemicals retention and satisfactory aromatic characteristics is required. Several researchers have reported on the degradation of phytochemicals upon thermal treatment and during heat treatment this loss might be due to harsh drying conditions, in particular, the temperature and duration used (Schieber, et al. 2001). Zainol, et al. (2009) discovered significant reductions of flavonoids in *Centella asiatica* during oven drying (76 to 97%) and vacuum drying (63 to 87%) as compared to freeze drying (31 to 73%). Similarly, Rabeta and Lin, (2015) also concluded that loss of total flavonoid, total phenol and antioxidant activities in the leaves and berries of *Cayratia trifolia* was found minimum during freeze drying as compared to vacuum oven drying. Sukrasno, et al. (2011) noticed that drying temperature influence flavonoid content of *Cosmos caudatus* (Kunth) leaves and highest content of flavonoids was retained in leaves dried at 40 ^{0}C while in lowest amount retained at 60 ^{0}C.

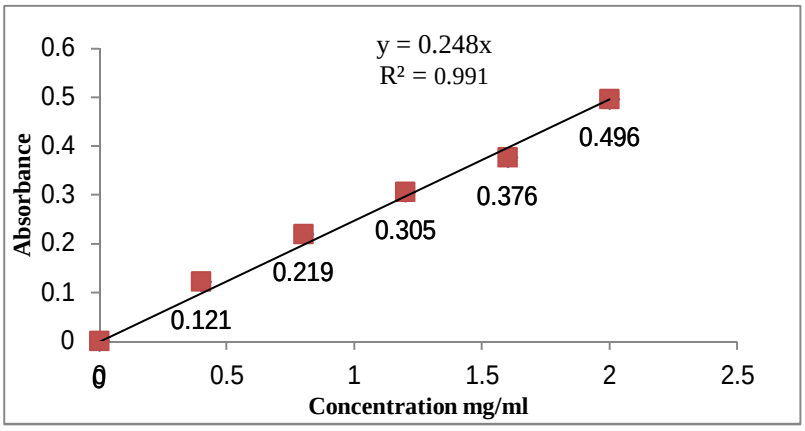

FIG. 1: QUERCETIN STANDARD CURVE

TABLE 2: TOTAL FLAVONOIDS CONTENT OF FREEZE DRIED AND TRAY DRIED POWDER OF PHALSA CULTIVARS

Drying method	Total flavonoids (mg of QE/ml of extract)	
	Tall cultivar	Dwarf cultivar
Freeze drying	0.396 ± 0.04	0.437 ± 0.02
Tray drying	0.235 ± 0.01	0.349 ± 0.04

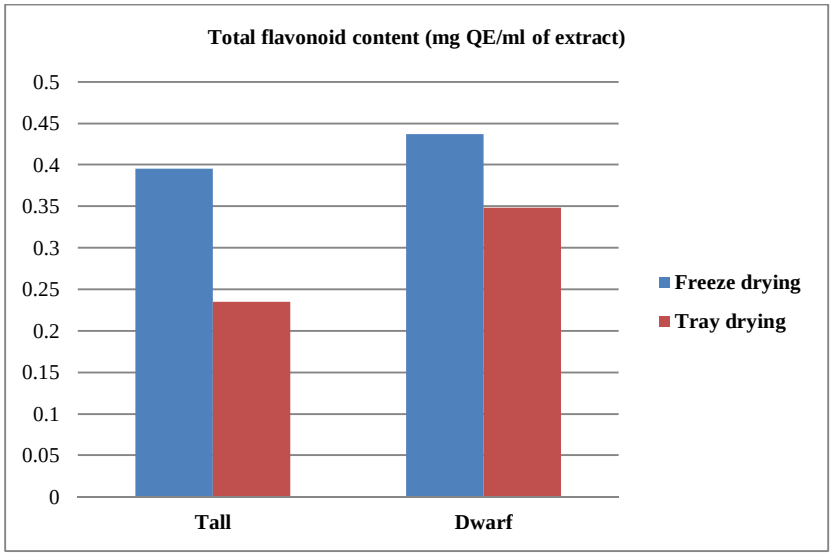

FIG. 2: TOTAL FLAVONOID CONTENT OF TRAY AND FREEZE DRIED POWDER OF PHALSA

Among various flavonoids quercetin and ellagic acid was found to have the highest thermal stability while gallic acid and caffeic acid exhibited the least thermal stability in sheep tallow olein as reported by (Elhamirad and Zamanipoor, 2012). Sharma and Patni, (2013) concluded that a significant amount of flavonoids was contained in leaves, stem and callus of *G. asiatica* which is responsible for their immense antioxidant potential. Irina and Mohamed, (2012) reviewed that drying method significantly affect the degradation of flavonoids and freeze-drying leads to minor losses as compared to hot air drying which is more aggressive and cause greater losses. Mansour, (2016) also concluded that dry processing significantly affect the phytochemical contents and air shade drying was found more better than air – sun and oven drying for retaining total phenolics and flavonoids of thyme vulgaris. Thus freeze drying can effectively keep the bioactive components such as flavonoids, phenolics, amygdalin, chlorophylls, and carotenoids, but the drying rate is slow.

IV. CONCLUSION

Results from the study showed that freeze dried dwarf cultivar of phalsa contain highest amount TFC followed by freeze dried tall cultivar. Thus freeze drying (non-thermal) treatment resulted in the lowest degradation of flavonoids as compared to that of tray drying (thermal) treatment. The present study is a step to the potential use of phalsa (an underutilized fruit) as an important antioxidant carrier in food and pharmaceutical industries. Also, there is further scope for the identification, quantification and purification of bioactive compounds which are responsible for various activities like antioxidant, antimalarial, radioactive etc. of this fruit.

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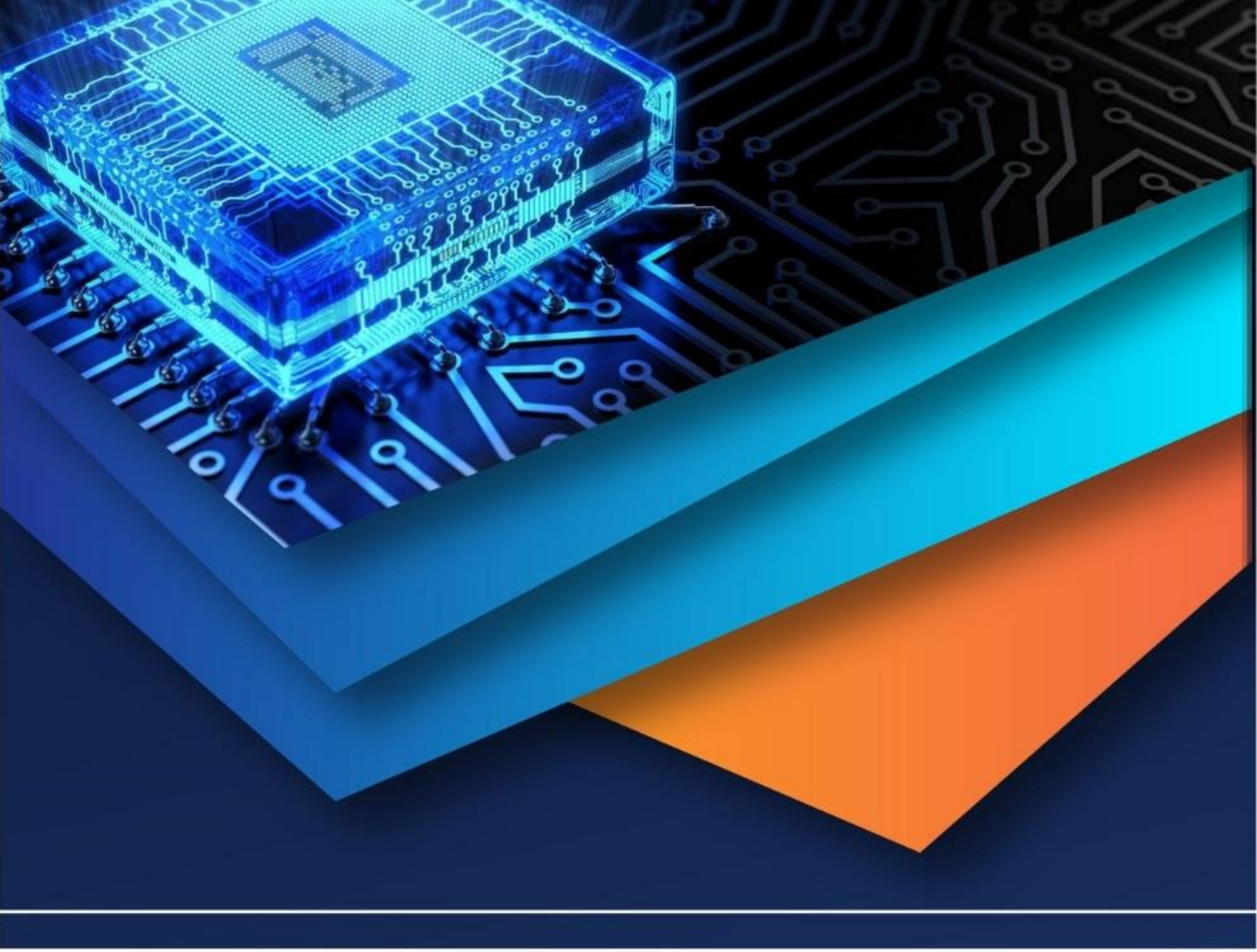

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