

Phenolic Contents and Antioxidants Activities in Jamman Fruit (*Eugenia jambolana*) Products

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Abstract: In this study, total phenolic contents and antioxidant activity were determined in various products made from two cultivars of jamman fruit (*Eugenia jambolana*) i.e. improved (V1) and indigenous (V2). Five products were made from each cultivar such as jam, squash, ready-to-drink juice, pulp powder and seed powder. Fruit cultivars were endogenous to the typical region of Sindh-Pakistan. Total phenolic contents were analyzed by Folin-Ciocalteu colorimetric method, whereas, the total antioxidant activity was analyzed by using the 2, 2-diphenyl-1-picrylhydrazyl radical scavenging capacity (DPPH) assays. The highest total phenolic contents and antioxidants were observed in freeze dried seed powder while the lowest contents were found in squash.

Keywords: Jamman fruit products, antioxidant capacity and total phenols.

INTRODUCTION

Plants are the potential source of natural phytochemical antioxidants which work as secondary metabolites of plants [1]. They are carotenoids, flavonoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid, tocopherols, tocotrienols etc. Their role in the sustenance of plant activity is also well documented [2]. The phenolic compounds and antioxidants from fruit play a pivotal role in human health and even pertinence of life is more or less dependent on them [3]. These constituents when included in food, lessen the risk of human diseases [4]. Antioxidants in lower concentrations delay and prevent the oxidation of substrate in a living system and help to sustain the fruit and/ or product for longer periods [5]. Antioxidants are added in foods to prevent or delay oxidation of food, initiated by free radicals formed during their exposure to environmental factors such as air, light and temperature [6]. Generation of free radicals or reactive oxygen during metabolism, exposure and other activities beyond the antioxidant capacity of a biological system leads to oxidative stress [7]. Oxidative stress plays a role in heart diseases, neurodegenerative diseases, cancer and in the aging process [8]. Oxidative damage in the human body plays an important causative role in disease initiation and progression [9]. Various researchers have already reported that consumption of fruits and vegetables to lower the risk for chronic diseases such as cancer,

cardiovascular diseases and stroke [10]. At the moment, most of the antioxidants manufactured are of synthetic origin. The biggest disadvantage with the synthetic antioxidants are the side effects when taken by a living entity [11].

E. jambolana (Jamman fruit), most of its components and products are used in traditional system of medicine. Its bark is digestive and astringent, anthelmintic, curative for sore-throat, bronchitis, asthma, quenching thirst, biliousness, dysentery, blood impurities and ulcers. In Unani system of medicine it or parts of it are given to patients as liver tonic to enrich blood, strengthen teeth and gums besides forming good lotion for removing ringworm infection in the head region. The vinegar prepared from the jamman fruit is tonic, astringent, carminative and useful in spleen diseases. The jamman seeds are also good for diabetes and suppressed urine conditions [12]. When externally used, jamman bark shows good wound healing [13]. In addition, Jamman's bark with or without the addition of other astringents like cardamom and cinnamon is also used as decoction in case of chronic diarrhea and dysentery. It also acts as a gargle in sore throat and spongy gums.

Furthermore, It has been observed that antioxidant activity of jamman fruit during oral administration of ethanolic extracts of jamman seed kernel to streptozotocin induced diabetic rats significantly decreased the levels of glycosylated hemoglobin, increased the body weight and hemoglobin, restored the activities of superoxide dismutase, catalase, glutathione peroxidase to the normal level. An increase in glutathione content and increased levels of lipid peroxidation and hydroperoxides in liver and kidney

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has also been reported in the study [14]. The same group later on in their studies reported similar findings in plasma and pancreas. They further revealed that jamman fruit had also the capacity to bring vitamin C, vitamin E and ceruloplasmin level to near normal in plasma [15].

MATERIALS AND METHODS

Chemicals and Reagents

Methanol (MeOH), Folin-Ciocalteu reagent, Gallic acid for standard curve, 2, 2-Diphenyl picryl hydrazyl (DPPH), Sodium carbonate (Na_2CO_3) were obtained of Sigma-Aldrich (St. Louis, USA). All chemicals were of analytical grade. Perkin-elmer UV-VIS lambda 25 Spectrophotometer was used to determine the absorbance.

Preparation of Jamman Fruit Samples

Two jamman fruit cultivars were used for making valuable products i.e. jam, squash, ready-to-drink juice, pulp powder and seed powder. Seed and pulp were dried through various methods i.e. freeze dried at (-50°C), Shade dried at ambient temperature ($32 \pm 2^\circ\text{C}$) and sun dried ($45 \pm 5^\circ\text{C}$) in solar chamber. Seed and pulp

was then ground to fine powder through grinder machine. The dried samples were packed into new glass jars and stored until further analyses were carried out.

Preparation of the Methanol Extracts

Samples weighing about 100g were extracted in a soxhlet with methanol (MeOH) at 60°C for 6 hours. The extract was then filtered and concentrated in vacuum at 45°C . Finally, the extracts were lyophilized and kept in the dark at 4°C prior to further testing.

Determination of Total Phenolic Compounds

The total phenolic content of the jamman fruit products were determined by the Folin-Ciocalteu method with some modifications. 5g per 50ml of sample was filtered with Whatman paper No.1. 0.5ml of the sample was added to 2.5ml of 0.2 N Folin-Ciocalteu reagents and placed for 5 minutes. 2ml of 75g/L of Na_2CO_3 were then added and the total volume was made up to 25 ml by using distilled water. The above solution was then kept for incubation at room temperature for 2 hours. Absorbance was measured at 765nm against a blank methanol sample using 1cm cuvette in a perkin-elmer UV-VIS lambda 25 spectrophotometer. Gallic acid was used to produce

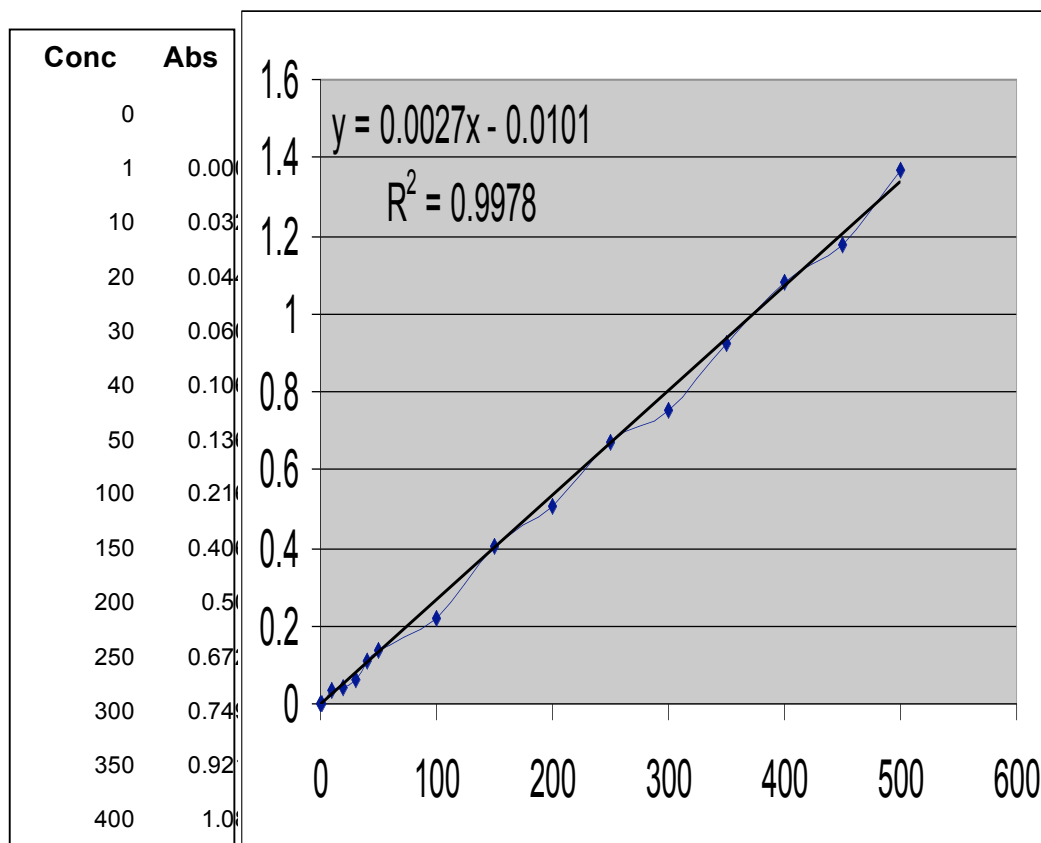


Figure 1. Standard graph of Folin-Ciocalteu colorimetric method.

standard calibration curve (Figure 1). The total phenolic content was expressed in mg of galic acid equivalents (GAE) /100g of lyophilized. The following formula was used to convert absorbance into ppm and subsequently into mg/g;

$$Y = MX + C \rightarrow X = \frac{Y + C}{M}$$

Where Y = absorbance and C & M values are obtained from standard curve.

Determination of Antioxidant Activity

The DPPH free radical method is based on the determination of the concentration of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) at steady state in a methanol solution, after adding the mixture of antioxidants. DPPH absorbs at 517nm and as its concentration is reduced by the existence of an antioxidant, the absorption gradually disappears with time. Briefly, 3.0ml of 1 mgml⁻¹ i.e. (60 X 10⁻⁶) concentrations of extracts from the Jamman product samples was added to 1.0 ml solution of DPPH radicals (0.1 mM, in 95% ethanol) and allowed to react at room temperature. The mixture was shaken vigorously through (Sonicor-shaker) and allowed to stand for 30 minutes and the absorbance of the resulting solution was measured at 517nm in a UV-VIS spectrophotometer. Lower absorbance of the reaction mixture indicated higher free-radical scavenging activity. The percent DPPH radical scavenging effect was calculated according to the following equation:

$$\text{DPPH scavenging effect (\%)} = \frac{[(AC(0) - AC(t)) / AC(0)] \times 100}{100}$$

Where AC(0) is the absorbance of the control DPPH solution at 0 min, and AC(t) is the absorbance in the presence of test samples at 20 min. The simplified shape for determining percent DPPH formula is as under

$$\% \text{ DPPH} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

The model of scavenging stable DPPH radicals is a widely used method for evaluating antioxidant activity in a relatively short time as reported by Fenglin *et al.*, [16]. The percent DPPH radical scavenging effect was calculated according to the equation as reported by Lo and Cheung [17].

RESULTS

Phenolic compounds as contained in various jamman fruit products studied are presented in Table-1. These products were observed to have the values of 11.155, 7.466, 17.212, 64.533, 50.173, 66.294, 3.078, 6.871 and 3.550 mg g⁻¹ total phenol in pulp powder shade dried, pulp powder sun dried, pulp powder freeze dried, seed powder shade dried, seed powder sun dried, seed powder freeze dried, squash, ready-to-drink juice and jam of V1, whereas 11.407, 7.488, 17.257, 64.705, 50.179, 67.688, 3.114, 6.922 and 3.596 mg g⁻¹ are found in pulp powder shade dried, pulp powder sun dried, pulp powder freeze dried, seed

Table 1: Determination of Total Phenolic Contents in Various Jamman Products

S.No.	Name of Sample	Absorbance (565.19 nm)	Quantity (mg/g)
1.	Pulp Powder Shade Dried V1 V2	0.29972	11.155c 11.407c
		0.30054	
2.	Pulp Powder Sun Dried V1 V2	0.12430	7.466c 7.488c
		0.12470	
3.	Pulp Powder Freeze Dried V1 V2	0.19507	17.212c 17.257c
		0.19524	
4.	Seed Powder Shade Dried V1 V2	0.89302	64.533c 64.705b
		0.89314	
5.	Seed Powder Sun Dried V1 V2	1.2034	50.173b 50.179 b
		1.20830	
6.	Seed Powder Freeze Dried V1 V2	1.1515	66.294a 67.688a
		1.15460	
7.	Squash V1 V2	0.045310	3.078d 3.114d
		0.045970	
8.	Ready-to-drink juice V1 V2	0.11367	6.871c 6.922c
		0.11450	

Table 2: Mean Antioxidant Activity in Various Jamman Fruit Products at 10 Minutes Interval

S. No.	Sample Name	Control	10min Absorbance	DPPH	% DPPH
01	Pulp powder shade dried V1 V2	0.64561 0.64561	0.42262 0.41451	0.3453943 0.35779	34.53943 35.779
02	Pulp powder sun dried V1 V2	0.64392 0.64392	0.4668 0.4458	0.2750652 0.307678	27.50652 30.7678
03	Pulp powder freeze dried V1 V2	0.64111 0.64111	0.34866 0.32936	0.456162 0.486266	45.6162 48.6266
04	Seed powder shade dried V1 V2	0.62605 0.62605	0.051165 0.041164	0.9182733 0.934248	91.82733 93.4248
05	Seed powder sun dried V1 V2	0.61129 0.61129	0.058956 0.047955	0.9035548 0.921551	90.35548 92.1551
06	Seed powder freeze dried V1 V2	0.059006 0.059006	0.049299 0.024079	0.9164509 0.959192	91.64509 95.9192
07	Squash V1 V2	0.55827 0.55827	0.32287 0.30487	0.4216598 0.453902	42.16598 45.3902
08	Ready-to-drink juice V1 V2	0.51942 0.51942	0.27959 0.25168	0.4617265 0.51546	46.17265 51.546
09	Jam V1 V2	0.61924 0.61924	0.52987 0.51081	0.1443221 0.175102	14.43221 17.5102

powder shade dried, seed powder sun dried, seed powder freeze dried, squash, ready-to-drink juice and jam of V2.

The mean antioxidant potency was determined through percent DPPH. The mean antioxidant activity at 10 minutes time interval was found to be 34.5394, 27.506, 45.616, 91.827, 90.355, 91.645, 42.165, 46.172, 35.779, 30.767, 48.626, 93.424, 92.155, 95.919, 45.390, 14.432 and 51.546% DPPH in pulp powder shade dried, pulp powder sun dried, pulp powder freeze dried, seed powder shade dried, seed powder sun dried, seed powder freeze dried, squash, ready-to-drink juice and jam respectively in V1 and V2 respectively (Table-2). The results of phenolic compounds are significantly higher in samples of seed powder which are shade dried, sun dried and freeze dried. It is equally true for their antioxidant activity which is also significantly higher and elevated in these samples of both the cultivars of jamman fruit. The greatest scavenging effects were found in freeze-dried seed powders while the lowest scavenging effect was observed in jam of varieties V1 and V2 respectively. Seed powder shade and sun dried remained second and third in DPPH scavenging effect of V1 and V2 respectively. The immediate next highest DPPH scavenging effect was noticed in pulp powder freeze dried, pulp powder shade dried and pulp powder sun dried in V1 and V2 respectively. Ready-to-drink juice

also showed greater scavenging activity than squash in varieties V1 and V2 respectively. The results of this study clearly state that seed and pulp powder through freeze drying have highly significant scavenging effect than other two methods. Similarly a highly visible difference between drying methods and similar differences were recorded between the products and varieties. V2 is relatively a better than V1 in DPPH scavenging effect. The results further indicated that there is co-relationship between total phenolic content and antioxidant activity among the Jamman fruit and its products, V2 however, showed more potency of phenolic compounds coupled with better DPPH scavenging effect.

DISCUSSION

The highest total phenolic contents were observed in freeze dried seed powder (66.294 and 67.688 mg gallic acid equivalent (GAE) g⁻¹ dry weight (DW), followed by shade and sun dried powders of seed (64.533 and 64.705) and (50.173 and 50.179) mg g⁻¹ of V1 and V2 respectively. While the lowest content were found in squash of both the cultivars such as 3.078 and 3.114 mg g⁻¹ which was followed by V1 Jam then V2 in increasing order (3.550 and 3.596 mg g⁻¹. Although, ready-to-drink juice has not enough quantity but still it was higher than squash and jam i.e. 6.871 and 6.922

mg g⁻¹. The pulp powder was ranked second highest after seed powder. Again, the freeze-dried pulp was higher than other methods (sun drying and shade drying). The freeze-dried pulp powder showed 17.212 and 17.257, shade dried 11.155 and 11.407 whereas sun dried pulp powder contained 7.466 and 7.488 mg g⁻¹. These results are in agreement with the findings of Zhi *et al.*, [18] who observed that the total phenolic content in the methanolic extract of jamman was 610.32mg/L while the flavonoid content was 451.50mg/L. These findings are also comparable with the amount of total phenolics in the blackberry fruit wines and liquors where they ranged from 91 to 1820mg GAE/L [19]. Further, these results are also comparable with earlier studies on horticultural crops, including raspberry, strawberry, pomegranate and blueberries as described by Kalt *et al.*, [20] and Ozgen *et al.*, [21]. The study further indicates that phenolic compounds are affected by processing and storage and these compounds remain more active in freezing temperatures than at higher temperatures. Joshi *et al.*, [22] reported decreases of phenolic contents in red wine during processing and four months storage from 68.9 to 53.4mg GAE/serving. Another study on the change of phenolic contents in blended and 23 months stored wine were also reported by Monagas *et al.*, [23]. These variations are possibly due to the transformation of phenolic compounds into their condensed forms in the product they studied which possesses slightly different chemical properties and reactivity's towards the Folin-Ciocalteu reagent [24].

The findings of the study are highly significant than earlier studies such as Li *et al.*, [25] who observed a wide variation in total phenolic content in jamman fruit, with a range of 5.18-8.53mg g⁻¹. Results of this study pointed out that there are statistically significant differences among all the products studied but only slight differences between the cultivars used. V2 is however, superior to V1 on phenolic composition. Phenolic compounds, such as quercetin, rutin, narigin, catechins, caffeic acid, gallic acid and chlorogenic acid, are very important phytochemical constituents because of their antioxidant activities [26]. Phenols play an indispensable role in human nutrition being very imperative plant constituents because of their radical scavenging ability due to their hydroxyl groups [27]. The phenolics content may contribute directly to the antioxidative action [28]. Further more, Shahidi and Wanasundara reported that phenolics behave as antioxidants, due to the reactivity of the phenol moiety [29]. It has also further been reported that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans [30]. Consequently, the antioxidant activities of plant/herb extracts are often explained by their total phenolics and flavonoid contents with fairly good correlation. These results

demonstrate that phenolic compounds represent the antioxidant activity in jamman fruit and its products. It is well-known that phenolic compounds contribute to fruit quality and nutritional value by modifying color, taste, aroma, and flavor and also by providing beneficial health effects. Mega reported that phenolic acids have been associated with color, sensory qualities, nutritional and antioxidant properties of foods [31]. These compounds also play a role in defensive mechanisms by counteracting reactive oxygen species, thus minimizing molecular damage due to microorganisms, insects and herbivores [32]. Phenols are very important plant constituents because of their radical scavenging ability due to their hydroxyl groups [16].

Determination of Antioxidant Activities

The antioxidant activities have also been investigated in the study by using DPPH method (Table 2, Figure 2).

The greatest scavenging effects were found in freeze-dried seed powders 91.64 and 95.91%, while the lowest scavenging effect was observed in jam with the values of 14.43 and 17.51% of V1 and V2 respectively. Seed powder shade and sun dried remained second 91.82 and 93.42% and third 90.35 and 92.15% in DPPH scavenging effect i.e. for V1 and V2 cultivars. The immediate next highest DPPH scavenging effect was noticed in pulp powder freeze dried, pulp powder shade dried and pulp powder sun dried with values of 45.61, 48.62, 34.53 and 35.77, 27.50652 and 30.76% of V1 and V2 respectively.

Ready-to-drink juice showed greater scavenging activity of 46.17 and 51.54% than squash 42.16 and 45.39% of V1 and V2 varieties. These findings are in line with the observations of Faiyaz *et al.*, [33] who reported that both untreated and heat treated extracts of jamman seed exhibited significant dose dependent radical scavenging activity ranging between 40-65%. Kurt *et al.*, [34] also reported 76.8mg/ml DPPH in jamman fruit aqueous extraction. Sandra *et al.*, on the other hand, reported 52mg/100g vitamin C content as DPPH scavenging activity in jambu pulp [35]. Where as, Bushra *et al.*, [36] noticed the DPPH radical scavenging activity of jamman bark which ranged between 49% to 87%. However, the gigantic difference among jam, ready-to-drink juice. The squash might be due to length of pasteurization during processing as ready-to-drink juice and squash is pasteurized at comparatively low temperatures for short time than jam which is almost cooked at high temperature which may have caused damage to the antioxidant activity. Besides, the results of this study clearly state that seed and pulp powder through freeze drying have highly

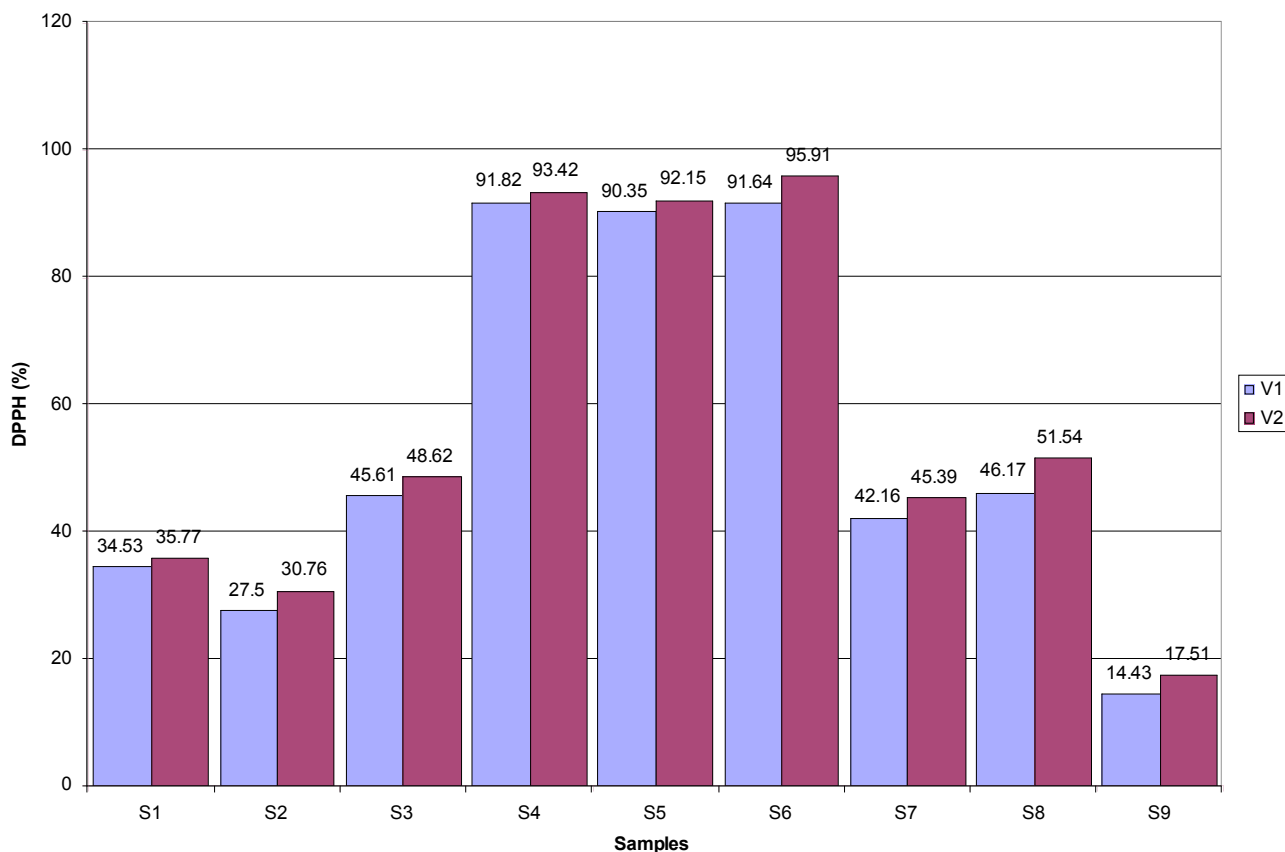


Figure 2: DPPH scavenging effect as percent antioxidant activity of various jamman fruit products. S1: Pulp powder shade dried **S2:** Pulp powder sun dried **S3:** Pulp powder freeze dried **S4:** Seed powder shade dried **S5:** Seed powder sun dried **S6:** Seed powder freeze dried **S7:** Squash **S8:** Ready-to-drink juice **S9:** Jam

significant scavenging effect than other two methods. Similarly a highly visible difference between drying methods and a similar differences were recorded between the products and cultivars. V2 is relatively a better than V1 in DPPH scavenging effect.

The results further indicate that there is a significant relationship between total phenolic content and antioxidant activity among the jamman fruit and its products, as V2 showed more potency of phenolic compounds coupled with better DPPH scavenging effect. These results are in agreement with similar other studies [25, 37]. Zhi *et al.*, also reported that antioxidant activity of jamman leaf extracts was related to their phenolic substrates [18]. The results indicated that the differences in total phenolic content and antioxidant activity among the jamman products were statistically significant and it is believed since all jamman products contain phenolic substrates hence their antioxidant activity may also be variable and different.

From the results of present study, it is adequately revealed that jamman fruit has a significant activity of antioxidants and phenolic compounds and their use in

fresh form or their products are equally beneficial to human health. The positive health effects may be due to high contents of certain phenolic compounds in plant-derived foods [38].

CONCLUSION

In pursuance of these results, it is concluded that both jamman fruit cultivars have a considerable quantity of phenolic compounds and antioxidants. The freeze drying method is more successful on retaining phenolic compounds and antioxidant activity than other two methods (sun drying and shade drying). Besides, seed powder and pulp powder have more antioxidant activity than the processed products like jam, squash and juice.

REFERENCES

- [1] Walton, NJ. and Brown, DE. 1999. Chemicals from plants: Perspectives on plant secondary products. London: Imperial College press.
- [2] Mccall, MR. and Frei, B. 1999. Can antioxidant vitamins materially reduce oxidative damage in humans?. Free radical Biology and Medicine. 26: 1034-1053.

- [3] Cao, GH., Alessio, HM. and Cutler, RG. 1993. Oxygen-radical absorbency capacity assay for antioxidants. *Free Rad. Biol. Med.* 14: 303-311.
- [4] Cilla, A., Laparra, JM., Alegria, A., Barbera, R. And Farre, R. 2008. Antioxidant effect derived from bioaccessible fractions of fruit beverages against H₂O₂-induced oxidative stress in Caco-2 cells. *Food Chemistry.* 106:1180-1187.
- [5] Halliwell, B. and Gutteridge, JMC. 1989. *Free radicals in biology and medicine* (2nd ed.). Oxford: Clarendon press.
- [6] Hras, AR., Hadolin, M., Knez, Z. and Bauman, D. 2000. Comparison of antioxidative and synergistic effects of rosemary extract with alpha-tocopherol, ascorbyl palmitate and citric acid in sunflower oil. *Food Chemistry.* 71: 229-233.
- [7] Zima, TS., Fialova, L., Mestek, O., Janebova, M., Crkovska, J., Malbohan, I., Strpek, S., Mikulikova, L. and Popov, P. 2001. Oxidative stress, metabolism of ethanol and alcohol-related diseases, *Jour. of Biomedical Sci.* 8: 59-70.
- [8] Astley, SB. 2003. Dietary antioxidants - past, present and future. *Trends in Food Sci. and Technol.* 14: 93-98.
- [9] Yamaguchi, T., Takamura, H., Matoba, T. and Terao, J. 1998. HPLC method for evaluation of the free radical-scavenging activity of foods by using 1,1-diphenyl-2-picrylhydrazyl. *Bioscience, Biotechnology and Biochemistry.* 62:1201-1204.
- [10] Yeum, KJ., Aldini, G., Chung, HY., Krinsky NI. and Russell, RM. 2003. The activities of antioxidant nutrients in human plasma depend on the localization of attacking radical species. *Jour. Nutr.* 133: 2688-2691.
- [11] Chen, C., Pearson, AM. and Gray, JI. 1992. Effects of synthetic antioxidants (BHA, BHT and PG) on the mutagenicity of IQ-like compounds. *Food Chemistry.* 43: 177-183.
- [12] Priyavtra, S. and Mehta, PM. 1969. In *Dravyaguna vignyan. Part II & III, (The Chowkhamba Vidyabhawan, Varansi)*, 586.
- [13] Nadkarni, KM. 1954. In *Indian Materia medica. Vol. I, (Popular book depot Bombay)*. 516-18.
- [14] Ravi, K., Ramachandran, B. and Subramanian, S. 2004a. Effect of *Eugenia jambolana* seed kernel on antioxidant defense system in streptozotocin-induced diabetes in rats. *Life Sciences.* 75 (22): 2717-2731.
- [15] Ravi, K., Ramachandran, B. and Subramanian, S. 2004b. Protective effect of *Eugenia jambolana* seed kernel on tissue antioxidants in streptozotocin-induced diabetic rats. *Biological & Pharmaceutical Bulletin.* 27 8: 1212-1217.
- [16] Fenglin, H., Ruili, L., Bao, H. and Liang, M. 2004. Free radical scavenging activity of extracts prepared from fresh leaves of selected Chinese medicinal plants. *Fitoterapia.* 75: 14-23.
- [17] Lo, KM. and Cheung, PCK. 2005. Antioxidant activity of extracts from the fruiting bodies of *agroclybe aegeria* var. *alba*. *Food Chemistry.* 89:533-539.
- [18] Zhi Ping Ruan., Liang Liang Zhang and Yi Ming Lin. 2008. Evaluation of the Antioxidant Activity of *Syzygium cumini* Leaves *Molecules.* 13: 2545-2556.
- [19] Heinonen, M., Lehtonen, PJ. and Hopia, AI. 1998. Antioxidant activity of berry fruit wines and liquors. *J. Agric. Food Chem.* 46: 25-31.
- [20] Kalt, W., Forney, CF., Martin, A. and Prior, RL. 1999. Antioxidant activity, vitamin C, phenolics, and anthocyanins after fresh storage of small fruits. *J. Agric. Food Chem.* 47: 4638-4644.
- [21] Ozgen, M., Durgaç, Serçe, C. and Kaya, C. 2008. Chemical and antioxidant properties of pomegranate cultivars grown in Mediterranean region of Turkey. *Food Chem.* 110: 703-706.
- [22] Joshi, VK. 1997. *Fruit Wine, Nauni, Solan (HP)*. Directorate of Extension Education, University of Horticulture and Forestry, Ch. 2.
- [23] Monagas, M., Nunez, V., Bartolome, B. and Gomez-cordoves, C. 2006. Phenolic content of blends of tempranillo with graciano or cabernet sauvignon wines produced in Spain. *Food Technol. Biotechnol.* (44): 507-513.
- [24] Singleton, VL. and Rossi, JA. 1965. Colourimetry of total phenolics with phosphomolibdic phosphotungstic acid reagent. *American Jour. Enol. Vitic.* (16): 144 –158.
- [25] Li, JW., Ding, SD. and Ding, XL. 2005. Comparison of antioxidant capacities of extracts from five cultivars of Chinese jujube. *Proc. Biochem.* 40: 3607-3613.
- [26] Paganga, G., Miller, N. and Rice-Evans, CA. 1999. The polyphenolic content of fruit and vegetables and their antioxidant activities. What does a serving constitute? *Free Radical. Res.* 30: 153-162.
- [27] Hatano, T., Edamatsu, R., Hiramatsu, M., Mori, A., Fujita, Y., asuhara, T. Yoshida, T. and Okuda, T. 1989. Effect of interaction of tannins with co-existing substances VI. Effect of tannins and related polyphenols on superoxide anion radical and on DPPH radical. *Chem. Pharm. Bull.* 37: 2016-2021.
- [28] Duh, PD., Tu, YY. and Yen, GC. 2004. Antioxidant activity of water extract of *Hargng Jyur (Chrysanthemum morifolium Ramat)*. *Leb-ensm. Wiss. Technol.* 32: 269–277.
- [29] Shahidi, F. and Wanasundara, PK. 1992. Phenolic Antioxidants. *Crit. Rev. Food Sci. and Nutr.* 32: 67-68.
- [30] Tanaka, M., Kuei, CW., Nagashima, Y. and Taguchi, T. 1998. Application of antioxidative maillard reaction products from histidine and glucose to sardine products. *Nippon Sui. Gakk.* 54: 1409-1414.
- [31] Mega, JA. 1978. Simple phenol and phenolic compounds in food flavor. *Crit. Rev. Food Agric.* 10: 323-372.
- [32] Vaya, JB. and Aviram, PAM. 1997. Antioxidant constituents from licorice roots: Isolation, structure elucidation and antioxidant capacity toward LDL oxidation. *Free Rad. Biol. Med.* 23: 302-313.
- [33] Faiyaz, A., Shivaprasad, H. and Malik, FA. 2010. In vitro study on the radical scavenging and anti lipidperoxidative effects of *Eugenia jambolana* aqueous extracts. *Jour. of Pharmacy Research.* 3: 198-200.
- [34] Kurt, AR., Basile MJ. and Kennelly, EJ. 2005. Antioxidant potential of seven myrtaceous fruits. On line: www.ethnobotanyjournal.org/vol3.
- [35] Sandra, AA., Velloso, JCR., Brunetti, IL., Khalil, NM., Silva, KM., Leite, C., Martins, ABG. and Oliveira, MMF. 2009. Antioxidant activity, ascorbic acid and total phenol of exotic fruits occurring in Brazil. *International Journal of Food Sciences and Nutrition.* <http://www.informaworld.com/smpp/title~db=all~content=t713425816~tab=issueslist~branches=60-v6060> (5): 439 – 448.
- [36] Bushra, S., Anwar F., and Przybylski, R. 2007. Antioxidant activity of phenolic components present in barks of *Azadirachta indica*, *Terminalia arjuna*, *Acacia nilotica*, and *Eugenia jambolana* Lam. trees. *Food Chemistry,* 104:1106-1114.
- [37] Yang, JH., Lin, HC. And Mau, JL. 2002. Antioxidant properties of several commercial mushrooms. *Food Chem.* 77: 229-235.
- [38] You, YL., Duan, XW., Wei, XY., Su, XG., Zhao, MM., Sun, J., Ruenroengklin N. and Jiang YM. 2007. Identification of major phenolic compounds of Chinese water chestnut and their antioxidant activity. *Molecules.* 12: 842-852.