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Stability assessment of Leh Berry fruit squash

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ABSTRACT

Leh berry fruit squash was optimized for shelf stability evaluation. The developed product was subjected to stability study by storing the product at two different temperature conditions viz., room temperature (23 ± 4°C) and 37°C. The various parameters viz. microbial, sensory and physico-chemical properties were analyzed. Shelf stability of squash showed the significant increase in total soluble solids, total sugars, reducing sugars and colour intensity for product stored at above temperature conditions except acidity and pH, which exhibited insignificant changes. The antioxidants *viz.*, vitamin C, E, phenols, anthocyanins and carotenoids showed significant reduction during entire storage period. The microbial population of fresh as well as seabuckthorn squash samples stored at above temperature conditions was found to be non-detectable. The sensory studies showed that the seabuckthorn squash was found to be acceptable up to 8 months at room temperature and 2 months at 37°C. Changes in the seabuckthorn squash had a significant effect on sensory attributes during storage for a period of 8 months at above temperature conditions. This product can be utilized throughout the year especially during the off season period and they can be safely consumed up to a period of 8-months without any further quality deterioration at room temperature.

Key words: *Hippophae rhamnoides*, shelf life, sensory evaluation, microbial study, physico-chemical changes.

INTRODUCTION

Leh berry commonly called as Sea buckthorn (*Hippophae rhamnoides*) belongs to family Elaeagnaceae, is a salt and drought tolerant deciduous shrub, which grows up to a height of four meter, widely distributed throughout continents of Asia and Europe (Lu, 13). It thrives well in sandy loam soil with an optimum pH of 6-7. They require good sunlight for its growth and they won't tolerate shade during its growth. It contains stiff and dense branches. The shrub starts to yield fruits, which starts from three months after pollination period (Li and Beveridge, 12). The shrub yields very tiny oval shaped orange colored fruits. The fruits were used as a food and pharmaceutical purpose in olden centuries, because of its potential therapeutic benefits. Several products like beverages, jam, food colours and oil can be derived from seabuckthorn berries.

In olden days, it was used for controlling fever, cleansing lungs, reducing inflammation, treating cold and coughs and also for suppressing tumor growth (Zeb, 23). The juice extracted from berries, which is being used as a health drink, which contains several phytoconstituents, especially rich source of antioxidants *viz*., vitamin C (500 mg/100g) and flavonoids (61 mg/100g) (Zhang *et al*., 24; Tong et al., 19; Wolf and Wegert, 22). The oil obtained from leh berry is extensively used as a topical agent for curing skin related problems.

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The fruits ripen and ferments fast, when it is exposed to environmental conditions, therefore it is essential to process the fruit into stable form i.e. by means of value addition as a squash. The reason for opting for processing the berries as squash is to preserve the juice for a prolonged period and also to stop further fermentation. The shelf stability of a product plays a crucial role in acceptance of the product after a prolonged storage period and it also helps in use of such product by consumers without any restriction. Therefore, the present study was undertaken to assess the shelf life of Leh berry fruit squash in order to preserve the various antioxidants and at the same time to benefit the consumers in terms of health benefits.

MATERIALS AND METHODS

Leh berry fruits were procured from Field Research Laboratory (Leh, India). The fruits were lifted through air and stored at frozen condition until its use. The ingredients used for preparing fruit squash *viz*., sugar, citric acid and preservative i.e. sodium benzoate were brought from local market.

The squash was developed as per the standard procedure of Girdharilal *et al*. (7). The frozen seabuckthorn berries were thawed to room temperature, sorted and washed to remove any adhering stalks and leaves, cleaned berries were partially crushed in mixer grinder, filtered through the stainless steel sieve having screen size of 30-mesh.

The fine pulp was pasteurized at 85°C for 30 min, blended with hot acidified sugar syrup as per the treatment (Table 1), packed hot in sterilized glass bottles, kept for stability study at various temperature conditions *viz*., room temperature (23 ± 4°C) and 37°C.

Product TSS (total soluble solids) was carried out using hand refractometer (Erma, Tokyo, Japan). The titratable acidity was carried out by titrating the sample with standard NaOH using phenolphthalein as an indicator (Ranganna, 14). The total and reducing sugar content of stored sample was evaluated as per the Fehling's method of Lane and Eynon (Ranganna, 14). The ascorbic acid content in sample was carried out using 2, 6-dichlorophenol-indophenol titration method (Ranganna, 14). The total carotenoids were determined by extracting the sample with hexane and acetone mixture followed by detecting at wave length of 450 nm by using UV spectrophotometer (UV 1601, Shimadzu Corp., Columbia, USA) (Arya *et al*., 2). The anthocyanin content was determined by extracting the sample with ethanolic HCl and detecting spectrophotometrically at 535 nm (Clydesdale and Francis, 4). The total phenol content was measured with the Folin-Ciocalteu's reagent spectrophotometrically as per the method of Singleton and Rossi (16). Vitamin E was estimated using Ferric Chloride-Dipyridyl, spectrophotometrically as per the protocol given by Desai (5). The intensity of browning in the stored sample was done as per the method of Verma and Sastry (20).

The fresh and stored product was evaluated after its reconstitution with water by a group of 20 judges, who had experience and exposure in evaluating the similar products. Hedonic rating scale ranging from 9: "extremely like" and 1: extremely dislike" was adopted to assess the stored products (Amerine *et al*., 1).

Table 1. Formulation of Leh Berry fruit squash**.

Treatment	Sugar syrup (°Brix)*	Citric acid (g)	Juice (ml)	Sodium benzoate
SQ_1	45	0.50	30	600 ppm
SQ_2	50	0.50	30	600 ppm
SQ_3	55	0.50	30	600 ppm
SQ_4	60	0.50	30	600 ppm
SQ_5	65	0.50	30	600 ppm

*For preparing sugar syrup 32 ml of water is used for all treatment combination with sugar incorporation @ of 28 g for SQ_1 treatment combination; 32 g for SQ_2 treatment combination; 39 g for SQ_3 treatment combination; 47 g for SQ_4 treatment combination; 56 g for SQ_5 treatment combination for 100 ml of squash.
**TSS of prepared squash was 50 ° Brix.

The microbial load in the stored squash samples was analyzed in the beginning stage and once in every 2 months' gap interval up to a period of eight months. The stored sample was analyzed for its total plate count (TPC), coliform, spores, yeasts and moulds by serial dilution technique as per the protocol given by Harrigan and McCance (8).

All analysis was carried out in triplicate. The analysis of variance (ANOVA) was used to interpret results with the help of Microsoft Excel 2000 (Microsoft Corp., Washington, USA).

RESULTS AND DISCUSSION

The analysis of pulp obtained from fresh seabuckthorn berries shows that, the pulp contains TSS of 8°brix, acidity of 2.4%, vitamin C of 510 mg/100g, Total sugars of 6.2g/100g. The Table 2 shows the sensory evaluation of fruit squashes developed from Leh berry fruit. The SQ_4 treatment combination accorded highest sensory score in terms of color, body, aroma, taste and overall acceptability. This may be because of optimum incorporation of sweetener in the form of syrup, which might have led to significantly increase the acceptability score to a greater extent. The poor score was recorded for treatment combination of SQ_1, which may be because of lesser degree of sweetness intensity in the blended syrup during squash preparation. Based on the overall acceptability rating, the SQ_4 treatment combination was further chosen for conducting stability studies at above temperature conditions.

The physico-chemical evaluation of seabuckthorn squash samples stored at different temperature was given in Fig. 1 (A-K). The initial total soluble solids of the squash 50.0°brix significantly increased ($P<0.05$) to 53.0 and 53.6° brix, after 8 months of storage at room temperature (23 ± 4°C) and at 37°C, respectively (Fig.1. A). Garande *et al*. (6), Sogi and Singh (17) and Khan *et al.* (11) also reported an increasing trend in total soluble solids of jamun squash, kinnow and

Table 2. Sensory evaluation* of various Leh berry fruit squashes.

Treatment	Color	Body	Aroma	Taste	Overall acceptability
SQ_1	6.8^{a}	7.0^{a}	6.4^{a}	6.7^{a}	6.7^{a}
SQ_2	7.0^{a}	7.4^{a}	6.6^{a}	6.9^{a}	6.9^{a}
SQ_3	7.8^{b}	7.6^{a}	6.9^{a}	7.2^{a}	7.4^{ba}
SQ_4	$8.6b^{c}$	8.9^{b}	8.5^{b}	9.0^{b}	8.8^{bc}
SQ_5	$8.0b^{c}$	8.9^{b}	8.2^{b}	8.7^{b}	8.4^{bc}

^{a,b,c,} Letters in the horizontal row with the same superscript do not differ significantly at $p<0.05$.
*Evaluation was carried out after 1:3 reconstitutions with water.

orange date blended squash during a period of 8, 4 and 3 months storage periods at above temperature conditions. The acidity of the squash was 1.20% at beginning and this slightly decreased to 1.17 and 1.15%, after 8 months of storage at room temperature and 37°C. The pH of the squash was 3.06 initially and this slightly increased to 3.09 and 3.11 after 8 months of storage at room temperature and at 37°C. The slight decrease in acidity and increase in pH was found to be non significant at level of (*P>0.05*) (Fig. 1B and C). This observation is in agreement with the results of Garande *et al*. (6), who have also reported a slight decrease in acidity and increase in pH of jamun squash during period of eight months storage at room temperature.

The initial total sugar content of the squash (49.51%) increased to 59.82 and 60.40% after eight months of storage at room temperature and at 37°C (Fig.1D). The reducing sugar content of the squash was 14.84% at earlier period and this increased to 20.96 and 21.63% after 8 months of storage at room temperature and at 37°C (Fig.1. E). The increase in total sugar and reducing sugar content of the stored samples were significant at level of P<0.05. Garande *et al*. (6) and Khan *et al*. (11) were also reported an increasing trend of both total and reducing sugars for jamun and orange date blended squash during a period of eight and three months storage at room temperature. The initial vitamin C content of the squash was 105.0 mg/ 100 g, significantly decreased (P<0.05) to 44.12 and 4.26 mg/ 100 g, after 8 months of storage at room temperature and at 37°C. The vitamin C loss accounted to 58% at room temperature and 96% at 37°C after 8 months of storage period (Fig. 1F). The vitamin E content of squash 45.72 mg/ 100 g at initial period which significantly decreased (p<0.05) to 27.43 and 22.41 mg/ 100 g at room and 37°C temperature conditions respectively, after 8 months of storage period (Fig. 1G). The loss was accounted to 40 and 51% under room temperature and 37°C respectively, after 8 months of storage. The total carotenoids content of seabuckthorn squash was 1.65 mg/ 100 g at beginning period and this decreased significantly (*P<0.05*) to 0.72 and 0.41 mg/ 100 g, after 8 months of storage at room temperature and 37°C. The loss in total carotenoids accounted for 56% under room and 72% at 37°C temperature conditions after 8 months of storage (Fig. 1H). The initial total anthocyanin content of squash 0.31 mg/ 100 g, decreased significantly (*P<0.05*) to 0.15 and 0.03 mg/ 100 g, after 8 months of storage under room temperature and 37°C. The loss in total anthocyanins accounted for 51% and 88% under room and at 37°C temperature conditions respectively, after 8 months of storage period (Fig. 1I). The total phenols content of the seabuckthorn squash was estimated to be 136.06 mg/ 100 g initially and this was found to decrease significantly (*P<0.05*) to 83.04 and 36.76 mg/ 100 g after 8 months of storage at room temperature and at 37°C (Fig. 1J). The loss in total phenols accounted for 39% and 73% under room temperature and at 37°C respectively, after 8 months of storage. Barwal *et al*. (3) and Khan *et al*. (11), who have also reported a decreasing trend of vitamin C in plum seasoned and orange date blended squash at room temperature during period of 6 and 3 months of storage periods; Andersson *et al*. (21) also reported similar losses in the concentration of tocopherols during the storage of blended beverages prepared from seabuckthorn berry, rose hip and white grapes at 22°C after 35 days; Verma and Sastry (20) also reported a loss in total carotenoids during the 6 months storage period of comminuted orange squash at similar temperatures; Kannan and Susheela Thirumaran (10) and Garande *et al*. (6) also reported a decreasing pattern of anthocyanin in jamun squash during a storage period of 6 months and 8 months at room temperature; Spanos *et al*. (18), who have also reported a similar loss in the phenolic composition during the storage of apple juice concentrates at 25°C after 9 months.

Colour intensity i.e. browning increased significantly (P<0.05) from 0.16 OD to 0.49 and 1.27 OD, after 8 months of storage at room temperature and at 37°C (Fig. 1K). Jain *et al*. (9) also observed an increase in optical density i.e. browning of different fruit squashes during the period of 12 months storage at room temperature.

The sensory scores of squash samples stored at room temperature (23 ± 4°C) and at 37°C were presented in Table 3. The seabuckthorn squash had an overall acceptability score of initially 8.2, on a nine-point Hedonic scale and a score of 6.0 at the end of the storage period was taken as the limit for assessing the shelf stability of the stored product. Seabuckthorn squash was found to be acceptable up to 8 months of storage at room temperature and 2 months at 37°C. Changes in the seabuckthorn squash had a significant effect ($p < 0.05$) on sensory attributes during storage period for 8 months at above temperature conditions. Kannan and Susheela Thirumaran (10), Singh *et al*. (15), Sogi and Singh (17) and Khan *et al*. (11) were also reported similar results of decrease in sensory scores of squash as a result of time advancement for the jamun squash, barhal squash, kinnow and orange date blended fruit squashes during a storage period of 6, 4 and 3 months at room temperature and 37°C, respectively.

The microbial population of fresh as well as seabuckthorn squash samples stored at room

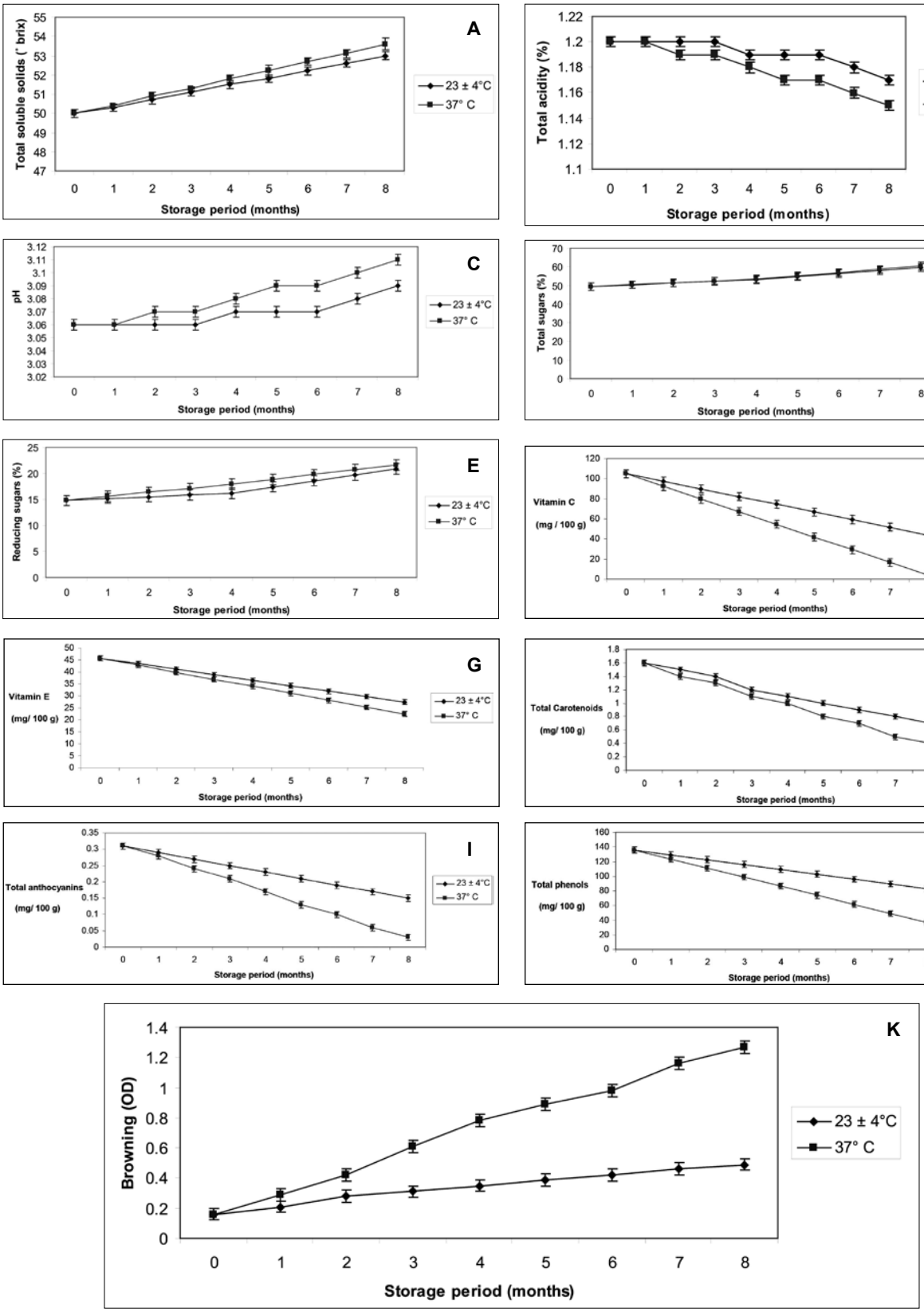

Fig. 1. (A-K). Physico-chemical evaluation of squash stored at different temperature.

Table 3. Sensory evaluation* of stored seabuckthorn squash (n=15).

Storage period in	Colour		Body		Taste		Aroma		Overall acceptability	
months	23 ± 4°C	37°C	23 ± 4°C	37°C	23 ± 4°C	37°C	23 ± 4°C	37°C	23 ± 4°C	37°C
0	8.3 ± 0.6	8.3 ± 0.6	8.0 ± 0.2	8.0 ± 0.2	8.4 ± 0.4	8.4 ± 0.4	8.1 ± 0.2	8.1 ± 0.2	8.2 ± 0.8	8.2 ± 0.8
1	8.1 ± 0.4	7.1 ± 0.4	7.9 ± 0.2	7.3 ± 0.8	8.1 ± 0.3	7.2 ± 0.3	7.9 ± 0.5	7.3 ± 0.4	8.0 ± 0.3	7.2 ± 0.7
2	7.8 ± 0.4	6.3 ± 0.2	7.6 ± 0.5	6.4 ± 0.3	7.9 ± 0.6	6.3 ± 0.4	7.7 ± 0.2	6.4 ± 0.9	7.7 ± 0.6	6.4 ± 0.5
3	7.6 ± 0.3	5.6 ± 0.6	7.3 ± 0.5	5.9 ± 0.5	7.7 ± 0.6	5.7 ± 0.2	7.4 ± 0.6	5.7 ± 0.4	7.5 ± 0.5	5.7 ± 0.4
4	7.3 ± 0.4	4.8 ± 0.8	7.0 ± 0.5	5.4 ± 0.2	7.5 ± 0.3	5.3 ± 0.7	7.1 ± 0.8	5.2 ± 0.2	7.2 ± 0.3	5.2 ± 0.6
5	7.0 ± 0.2	4.3 ± 0.5	6.8 ± 0.2	5.1 ± 0.3	7.2 ± 0.1	5.0 ± 0.4	6.8 ± 0.7	4.8 ± 0.5	6.9 ± 0.5	4.8 ± 0.5
6	6.7 ± 0.6	4.0 ± 0.5	6.5 ± 0.5	4.7 ± 0.9	6.8 ± 0.2	4.7 ± 0.5	6.5 ± 0.3	4.5 ± 0.6	6.6 ± 0.3	4.5 ± 0.3
7	6.2 ± 0.5	3.8 ± 0.6	6.3 ± 0.5	4.4 ± 0.3	6.5 ± 0.4	4.3 ± 0.2	6.2 ± 0.6	4.2 ± 0.7	6.3 ± 0.8	4.2 ± 0.6
8	6.0 ± 0.6	3.4 ± 0.7	6.0 ± 0.5	4.2 ± 0.3	6.1 ± 0.9	4.0 ± 0.6	6.0 ± 0.7	4.0 ± 0.3	6.0 ± 0.6	3.9 ± 0.5

*Values are mean ± standard deviation

temperature (23 ± 4°C) and at 37°C for a period of 8 months was found to be non detectable. These findings are in conformity with the reported results of Kannan and Susheela Thirumaran (10), who have also observed nil microbial growth for fresh and stored jamun fruit squash sample upto a period of 6 months at room temperature condition.

The SQ_4 treatment combination showed highest organoleptic acceptability, this may be because of use of optimum sweetening agent in the formulation. The studies indicated that the antioxidant rich squash can be developed naturally from wonderful fruit of Leh berry. This product can be utilized throughout the year especially during the off season period because of its processed form by means of value addition. It can be recommended as an antioxidant booster for the consumers in health point of view because of rich source of antioxidants like vitamins and polyphenols. The product stored at room temperature (23 ± 4°C) showed higher stability upto a period of 8-months. Therefore, the product can be safely consumed upto a period of 8-months without any deterioration in its quality at room temperature condition.

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REFERENCES

1. Amerine, M.A., Pangborn, R.M. and Roessler, E.B. 1965. *Principles of Sensory Evaluation of Food*. Academic Press, New York, USA.

2. Arya, S.S., Natesan, V., Parihar, D.B. and Vijayaraghavan, P.K. 1979. Stability of carotenoids in dehydrated carrots. *J. Food Technol.* **14:** 579-86.

3. Barwal, V.S., Sharma, R. and Lal, B.B. 2002. Use of sorbitol for the preparation of plum seasoned squash. *J. Food Sci. Technol*. **39:** 413 – 17.

4. Clydesdale, F.M. and Francis, F.J. 1976. Pigments pp. 385-426. **In**: *Food Chemistry,* O.R. Fennema, Marcel Dekker, New York, USA.

5. Desai, I.D. 1984. Improved spectrophotometric assay for vitamin E: oxygen radicals in biological systems pp. 142-144. **In**: *Methods in Enzymology*, L. Packer, Academic Press, Orlando, USA.

6. Garande, V.K., Joshi, G.D. and Waskar, D.P. 1995. Storage of jamun (*Eugenia cuminii* Druce) fruit products. *ASEAN Food J.* **10:** 54-56.

7. Girdharilal, G., Sidappa, S. and Tandon, G.L. 1986. *Preservation of Fruits and Vegetables*, Indian Council of Agricultural Research Publications, New Delhi, India.

8. Harrigan, W.F. and McCance, M.E. 1976. *Laboratory Methods in Food and Dairy Microbiology*. Academic Press, London, p. 214-215.

9. Jain, S.P, Tripathi, V.K., Ram, H.B. and Singh, S. 1986. Effect of storage conditions on storage life of some important squashes. *Indian Food Packer*, **40:** 36-41.

10. Kannan, S. and Susheela Thirumaran. 2004. Studies on the storage life of jamun (*Syzygium*

cuminii Rom.) fruit products. *J. Food Sci. Technol*. **41:** 186 – 88.

11. Khan, M.A., Hashmi, M.S., Muhammad, A., Muneeb, M., Bilal, H. and Wali, G. 2018. Development and storage study of orange date blended squash. *Sarhad J. Agric*. **34:** 509-15.

12. Li, T.S.C. and Beveridge, T.H.J. 2003. Sea Buckthorn (*Hippophae rhamnoides* L.): production and utilization. NRC Research Press, Ottawa. (www.monographs.nrc-cnrc.gc.ca).

13. Lu, R. 1992. Sea buckthorn: A multipurpose Plant Species for Fragile Mountains, International Centre for Integrated Mountain Development, Kathmandu, Nepal. pp. 1-62.

14. Ranganna, S. 1986. *Handbook of Analysis and Quality Control for Fruit and Vegetable Products*, Tata McGraw-Hill, Delhi, India.

15. Singh, I.J., Singh, H.K. and Chopra, C.S. 2004. Studies on storage stability of barhal (*Artocarpus lakoocha*) products. *Beverage and Food World*, **31:** 29-30.

16. Singleton, V.L. and Rossi, J.A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American J. Enol. Viticul.* **16:**144.

17. Sogi, D.S. and Singh, S. 2001. Studies on bitterness development in kinnow juice, ready–to–serve beverage, squash, jam and candy. *J. Food Sci. Technol.* **38:**433-38.

18. Spanos, G.A., Wrolstad, R.E. and Heatherbell, D.A. 1990. Influence of processing and storage on the phenolic composition of apple juice. *J. Agric. Food Chem.* **38:** 1572 – 79.

19. Tong, J., Zhang, C., Zhao, Z., Yang, Y. and Tian, K. 1989. The determination of the physical–chemical constants and sixteen mineral elements in raw sea buckthorn juice. *Proceedings of International Symposium on Sea buckthorn (H.rhamnoides L.),* Xian, China, pp. 132 – 37.

20. Verma, A.K. and Sastry, L.V.L. 1969. Preparation, composition and storage behavior of comminuted orange and lime squashes. Indian Food Packer, **23:**5-11.

21. Adersson, S.C., Ekholm, A., Johansson, E., Olsson, M.E., Sjoholm, I., Nyberg, L., Nilsson, A. and Rumpunen, K. 2015. Effect of storage temperature and time on stability of bioactive compounds in aseptically packed beverages prepared from rose hip and seabuckthorn berries. AGR. Food Sci. **24**: 273-88.

22. Wolf, D. and Wegert, F. 1993. Experience gained in the cultivation, harvesting and utilization of sea buckthorn pp. 23-29. **In**: *Cultivation and Utilization of Wild Fruit Crops*, Germany Bernhard Thalacker Verlag GmbH & Co, Germany.

23. Zeb, A. 2004. Important therapeutic uses of sea buckthorn (Hippophae): a review. *J. Biol. Sci*. **4:** 687-93.

24. Zhang, W., Yan, J., Duo, J., Ren, B. and Guo, J. 1989. Preliminary study of biochemical constituents of sea buckthorn berries growing in Shanxi province and their changing trend. *Proceedings of International Symposium on Sea buckthorn* (*H. rhamnoides* L.), Xian, China, pp. 96-105.

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