

Applied Biological Research 22(1): 10-19; (2020) DOI: 10.5958/0974-4517.2020.00002.6

# EFFECT OF HYDROCOLLOIDS AND STORAGE ON PHYSICOCHEMICAL, PHENOLIC AND ANTIOXIDANT PROPERTIES OF SEA BUCKTHORN SQUASH

Ursila Irshad, Amir Gull, Sajad Mohd Wani<sup>2</sup>\*, Farooq A. Masoodi, Tariq A. Ganaie<sup>3</sup> and Nusrat Jan<sup>2</sup>

Department of Food Science and Technology, University of Kashmir, Hazratbal, Srinagar - 190 006, Jammu & Kashmir (India)

<sup>2</sup>Division of Food Science and Technology, S.K. University of Agricultural Sciences & Technology of Kashmir, Shalimar, Srinagar – 190 025, Jammu & Kashmir (India)

<sup>3</sup>Department of Food Technology, Islamic University of Science and Technology, Awantipora

- 192 122, Jammu & Kashmir (India) \*e-mail: wanisajad82@gmail.com

(Received 22 August, 2019; accepted 27 December, 2019)

# ABSTRACT

The influence of three hydrocolloids namely xanthan gum, pectin, and guar gum at concentrations of 1.5% were studied to assess the cloud stability of sea buckthorn squash. The prepared squash was subjected to physicochemical, phenolic, antioxidant and sensory evaluation at 0, 30, 60 and 90 days of storage. In general, there was an increase in TSS, reducing and total sugars while decrease in acidity, ascorbic acid, carotenoids, phenolic compounds and antioxidant activity was observed in prepared sea buckthorn squash than pulp. During storage, sea buckthorn squash showed increase in acidity, TSS, reducing and total sugars in all the treatments. The squash containing pectin showed the high ascorbic acid content and squash containing guar gum showed high carotenoid contents at the end of storage period. The prepared squash treated with pectin and guar gum showed slight decrease in antioxidant activity while as high loss of antioxidant activity was observed in xanthan gum treatment at the end of storage. A declining trend was also observed in phenolic compounds in all the treatments during storage. HPLC analysis revealed that three phenolic compounds were present in pulp which was significantly (p<0.05) reduced in all the treatments in squash during storage period. Sea buckthorn squash containing pectin showed higher color and taste values followed by guar gum and xanthan gum during storage.

Keywords: Antioxidants, ascorbic acid, HPLC, reducing sugars, sea buckthorn

# INTRODUCTION

Sea buckthorn (*Hippophae rhamnoides* L., family Elaeagnaceae) is a deciduous, thorny willow-like plant species. Sea buckthorn is native to Asia and very large Eurasian area at different altitudes. It is a unique plant currently domesticated in different countries particularly China, Russia, Finland, Germany, France, Romania, India, Pakistan, Nepal, etc. (Selvamuthukumaran *et al.*, 2007). Sea buckthorn, also called the treasure of bio-activity substance, is rich in source of vitamin C, B<sub>1</sub>, B<sub>2</sub>, folic acid, organic acids, amino acids, tocopherols, flavanoids, phenols, terpenes and tannins (Li and Wang, 1998). Its berries contain a large amount of sugars especially glucose, fructose and organic acids, *viz*.,

malic, oxalic and tartaric acid (Kumar *et al.*, 2011). It is rich in carotenoids like zeaxanthin, carotene, cryptoxanthin, lutein, lycopene, etc. (Anderson *et al.*, 2009). Ascorbic acid acts as a biological antioxidant and its concentration in fresh sea buckthorn berries ranges between 200 to 400 mg 100 g<sup>-1</sup> (Rousi and Aulin, 1997). Because of rich source of phytochemicals, sea buckthorn is known to have several beneficial health effects, such as reduction of cardiovascular risk factors, treatment of gastrointestinal ulcers, therapy of skin disorders and remedy for liver cirrhosis (Eccleston *et al.*, 2002; Saggu *et al.*, 2007). These beneficial effects have made sea buckthorn products valuable for medicinal and cosmetic purposes.

Hydrocolloids are water soluble substances that have the ability to form gels when in contact with water. Hydrocolloids are used in food applications because of their novel characteristics such as thickener, gel, syneresis control, stabilizing emulsifier or suspension, as a coating and water binder (Sadar, 2004). Hydrocolloids usually used in food industry are carboxymethyl cellulose, starch, pectin, guar gum, xanthan gum, gelatin, etc. (Durand *et al.*, 2003). Guar gum act as a thickener, and used in sauces, gravies, and act as moisture retainer in cake, pie, donut and frozen foods (Labuza, 2011). Xanthan gum is stable over a wide range of pH from 2 to 12 which has water binding capacity and highly soluble in cold or hot water (Santos *et al.*, 2000; Labuza, 2011). The pectin extracted from plant cell wall is the most widely used as gelling and thickening agent in food. In jam and jellies pectin is usually used as a thickening agent to change the texture or flow behaviour of final product (Javanmard and Endan, 2010).

Some fruit products with pulp may precipitate after prolonged storage, and it is difficult to maintain a homogenous suspension of fruit pulp. Hydrocolloids are incorporated in food products to maintain turbidity and suspension in order to avoid separation. However, one of the main problems with sea buckthorn squash is that even after prolonged storage, none or only a few smart cloud particles should precipitate. The perusal of literature reveals that no report is available on the effect of using hydrocolloids such as pectin, guar gum or xanthan gum gelling agent on sea buckthorn squash. The present study was aimed to stabilize sea buckthorn squash by using hydrocolloids and evaluate the effects of storage on physicochemical, phenolic, antioxidant and sensory properties of squash.

# **MATERIALS AND METHODS**

#### **Materials**

Ripe sea buckthorn (*Hippophae rhamnoides* L.) berries were procured from Ladakh and kept frozen at -20°C till further analysis. Pectin, guar gum and xanthan gum (Hi-Media) were used as stabilizers. All the chemicals used in the present work were of analytical grade.

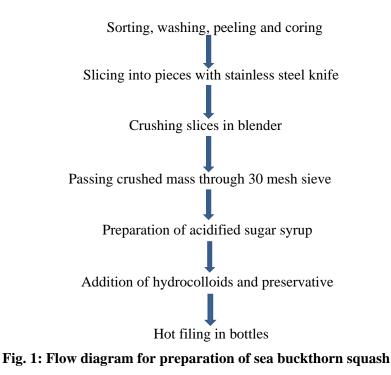
### Preparation of sea buckthorn pulp

The defective, injured and malformed sea buckthorn berries were excluded and ripe ones were retained for pulp extraction. The berries were washed under running tap water to remove any dirt, then crushed in an electric grinder-cum-mixer and strained properly using stainless steel sieve 30 mesh. The pulp obtained was kept under refrigeration till further use.

# Development of sea buckthorn squash

The flow sheet for preparation of sea buckthorn squash is presented in Fig. 1. Squash was prepared by mixing sea buckthorn pulp, sugar syrup and citric acid. The final squash characteristics were adjusted to 1.0 % acidity and 45% TSS. Hydrocolloids *viz.*, xanthan gum, pectin and guar gum at 1.5% concentration were added to sea buckthorn squash for cloud stabilization. Sodium benzoate (600 ppm) was added as preservative during product preparation. The squash was then hot filled in pre-cleaned, sterilized glass bottles, capped, sealed, labeled and stored for 90 days under ambient conditions.

Ursila Irshad et al.



# Physiochemical analysis

<u>Total soluble solids</u>: A hand refractometer (Abbes refractometer) was used to determine total soluble solids (TSS) as per the procedure given by AOAC (2005).

<u>Titratable acidity:</u> Titratable acidity was determined as per the procedure given by (Ranganna, 1986). Acidity was calculated as citric acid by using following formula:

 $Acidity\% = \frac{\text{Titre value} \times \text{normality of NaOH} \times \text{eqivalent weight} \times \text{volume made} \times 100}{\text{Weight of sample} \times \text{volume of aliquot} \times 1000}$ 

**<u>Reducing sugars:</u>** Reducing sugars was estimated by the Lane and Eynon method (Ranganna, 1986). For this, 10 g sea buckthorn was crushed and mixed with distilled water and volume made upto 100 mL in a volumetric flask and neutralized with 1 N NaOH. After this, the final volume of solution was made to 250 mL. To this solution, 2 mL of 45% lead acetate and 22% of potassium oxalate was added to precipitate the excessive lead acetate and filtered. Then 50 mL filtrate was taken and titrated with Fehling's solution (A and B) on a hot plate till brick red colour appeared. At this point methylene blue was added as an indicator and the total volume of sample consumed during titration was noted.

**Total sugars:** Total sugars were determined as per Lane and Eynon method (Ranganna, 1986). Aliquot of 50 mL sea buckthorn was taken and 50 mL water and 5 g citric acid added to it. The solution was neutralized with NaOH and then titrated with Fehling's solution as mentioned above. Total sugars were calculated using the formula:

% reducing sugars/ total sugars =  $\frac{\text{Fehling factor x volume made x 100}}{\text{Titer value x weight of sample}}$ 

<u>Total carotenoids and ascorbic acid</u>: Total carotenoids were analyzed as per the method of Rangana (1986). Absorbance readings were performed using spectrophotometer at 452 nm. Ascorbic acid was measured through titration method using 2, 6-dichlorophenol-indophenol. The results were expressed in terms of mg 100 g<sup>-1</sup>.

<u>Antioxidant activity (DPPH)</u>: Antioxidant activity was assayed by DPPH (2, 2-diphenyl-1-picryl hydrazyl) method (Brand-Williams *et al.*, 1995) using spectrophotometer. The absorbance was measured at 517 nm. The radical scavenging activity (antioxidant activity) was expressed as percent inhibition of DPPH radical:

### % I = $[(AB - AA) / AB] \times 100$

Where, AA is absorbance after sample incubation; and AB is the absorbance before sample addition

<u>Total phenolic content</u>: Total phenolic content was assayed spectrophotometrically by using Folin-Ciocalteu's reagent as per the procedure of Sharma and Gujral (2010). Total phenolic contents were expressed in terms of mg gallic acid equivalent  $100 \text{ g}^{-1}$  fresh matter (mg GAE 100 g<sup>-1</sup>).

# Determination of phenolic compounds by HPLC

This method was based on Abad-Garcia *et al.* (2007) with slight modification. To characterize the polyphenols compounds, reverse phase HPLC with photodiode array detection (DAD) was used based on their ultra-violet spectra. All polyphenol standard solutions (ranging from 25 to 250  $\mu$ g mL<sup>-1</sup>) were prepared in methanol and stored at 4°C in darkness. Chromatographic analysis was performed by HPLC system, Jasco Corporation (Tokyo, Japan), equipped with a DAD detector, and controlled by Empower software. An Atlantis C18 (150 × 4.6 mm) column with mobile phases A (acetic acid-water, 0.5: 99.5, v/v) and B (methanol) were used. The applied elution condition was in gradient mode, with a flow rate of 1.0 mL min<sup>-1</sup> and injection volume of 10  $\mu$ L. Phenolic compounds were monitored and quantified at 280 nm.

#### Sensory evaluation

Sensory analysis of fresh sea buckthorn squash was carried out as well as the squash stored for 90 days using 9-point Hedonic scale by semi-trained panel of 15 members. The trained taste panel consisted of the staff members at the Department of Food Technology, SKUAST. The squash was evaluated for sensory parameters such as colour and taste. Potable water was used for rinsing the mouth between testing the samples.

### Statistical analysis

All the analyses were carried out in triplicates and their mean values calculated. The significant differences were obtained using SPSS Statistics (v. 250 16, Inc., Chicago, USA for one-way analysis of variance, followed by Duncan's multiple range test ( $p \le 0.05$ ).

# **RESULTS AND DISCUSSION**

#### **Physicochemical properties**

Total soluble solids (TSS) represent various organic acids present in soluble form. TSS was significantly (p < 0.05) higher in squash in all three treatments as compared to the pulp (Table 1). The increase in soluble solids was because of the incorporation of solids during squash preparation. During storage, TSS content in squash showed significant (p < 0.05) increase in all treatments (Table 2). However, the addition of 1.5% xanthan gum caused a significant (p < 0.05) increase in TSS content (45.0-55.5° Brix) during storage. Increase in TSS appears due to the hydrolysis of polysaccharides to simpler sugars during storage. Similar findings were reported in squash prepared from water melon (Swamy *et al.*, 2011).

The acidity of a food product represents the stability and shelf life of product. Acidity value of squash is due to the organic acids naturally present in sea buckthorn fruit. The titratable acidity in squash significantly (p < 0.05) decreased in all the treatments as compared to pulp (Table 1). However, the acidity in squash increased significantly (p < 0.05) during storage period of 90 days in all the treatments (Table 2). Incorporation of 1.5% pectin caused slight increase in acidity (1.0-1.7%) among all the three treatments during storage. Increase in acidity in all the three treatments might be because of the oxidation of reducing sugars, acid formation and degradation of polysaccharide or by breaking down of uronic acid and pectin substances (Iqbal *et al.*, 2001; Hussain *et al.*, 2008). Similar findings were reported by Swamy *et al.* (2011) for watermelon squash.

Table 1. I hysiochennear characteristics of fresh sea buckhorn puip and squash					
Parameters	Fresh pulp	Squash T1	Squash T2	Squash T3	
TSS (°Brix)	$10.03^{a} \pm 2.5$	$45.0^{b}\pm4.0$	$45.0^{bc} \pm 4.0$	$45.0^{\circ}\pm4.0$	
Titratable acidity (%)	2.1 <sup>a</sup> ±0.9	$1.0^{c}\pm0.00$	$1.0^{b}\pm0.01$	$1.0^{bc} \pm 0.01$	
Total sugar (%)	$4.24^{b}\pm1.4$	$42.8^{\circ}\pm5.1$	$37.5^{ab}\pm2.8$	40.3 <sup>a</sup> ±4.2	
Reducing sugar (%)	$1.76^{a} \pm 0.1$	$33.8^{b}\pm2.2$	$28.6^{\circ}\pm2.8$	$32.5^{ac}\pm 2.8$	
Ascorbic acid (mg 100 g <sup>-1</sup> )	$228.3^{b} \pm 10.6$	$150.3^{a} \pm 10.1$	150.2 <sup>bc</sup> ±10.2	148.2°±10.2	
Carotenoids (mg 100 g <sup>-1</sup> )	75.3°±0.9	72.0 <sup>a</sup> ±7.1	$72.4^{b}\pm 6.1$	73.3 <sup>ab</sup> ±6.2	
Antioxidant (% inhibition)	87.15 <sup>a</sup> ±6.1	74.3 <sup>ac</sup> ±6.2	85.4 <sup>b</sup> ±7.3	85.4 <sup>c</sup> ±6.3	
TPC (mg GAE 100 g <sup>-1</sup> )	$6.12^{a}\pm2.0$	$3.2^{ab}\pm0.5$	3.4 <sup>b</sup> ±0.2	3.6°±0.1	
Protocatechinic acid (mg kg <sup>-1</sup> )	38.99 <sup>b</sup> ±5.0	$28.99^{a} \pm 4.8$	29.5°±3.5	$27.9^{ac} \pm 3.8$	
Quercetin (mg kg <sup>-1</sup> )	$13.19^{ab}\pm1.5$	$11.5^{b}\pm 1.2$	$11.4^{c}\pm 1.3$	$11.5^{ac} \pm 1.1$	
Kaempferol (mg kg <sup>-1</sup> )	$13.10^{a} \pm 1.0$	7.4 <sup>ab</sup> ±1.3	$7.8^{c}\pm1.1$	$7.8^{ac} \pm 1.2$	

Table 1: Physiochemical characteristics of fresh sea buckthorn pulp and squash

The values indicate mean  $\pm$  standard deviation. The values with different lower-case letters in each column are significantly (p  $\leq$  0.05) different from each other; T1: Sea buckthorn squash with xanthan gum; T2: Sea buckthorn squash with pectin; T3: Sea buckthorn squash with guar gum

Reducing sugars significantly increased in sea buckthorn squash as compared to the pulp (Table 1). This might be due to the inversion of non-reducing sugars into reducing sugars (Shreshta and Bhatia, 1982). In addition, total sugars were also found significantly (p < 0.05) higher in sea buckthorn squash than pulp which may be attributed to the partial hydrolysis of complex carbohydrates to sugars (Barwal and Shrera, 2009). Similarly, significant increase in reducing sugars and total sugar contents of sea buckthorn squash was observed in all the treatments during storage (Table 2). However, the addition of 1.5% guar gum showed significant increase in reducing sugars (32.5-47.1%) and total sugars (40.3-65.5%) among all the treatments during storage. Similar increases in total sugar content of squash incorporated with mango juice has been reported by Deka and Sethi (2001).

Table 2: Effect of hydrocolloids an	d storage on physicocl	hemical properties of sea	u buckthorn squash
-------------------------------------	------------------------	---------------------------	--------------------

		Storage period				
Hydrocolloid	Conc.	0 day	30 days	60 days	90 days	
Treatments	(%)	0 day	50 days	00 days	J0 days	
		Ac	<u>idity (%)</u>			
Xanthan gum (T1)	1.5	$1.0^{b}\pm0.00$	$1.4^{a}\pm0.01$	$1.6^{a}\pm0.03$	$2.0^{bc} \pm 0.04$	
Pectin (T2)	1.5	1.0a±0.01	$1.3^{b}\pm0.01$	$1.0^{b}\pm0.02$	1.7 <sup>b</sup> ±0.03	
Guar gum (T3)	1.5	$1.0^{bc} \pm 0.01$	$1.2^{bc} \pm 0.00$	$1.5^{bc} \pm 0.05$	$2.6^{a}\pm0.05$	
		TS	S (°Brix)			
Xanthan gum (T1)	1.5	45.0 <sup>b</sup> ±4.0	$45.5^{a} \pm 4.1$	$50.5^{a}\pm5.8$	$55.5^{\rm a}\pm5.5$	
Pectin (T2)	1.5	$45.0^{\mathrm{a}}\pm4.0$	$45.5^{b}\pm4.0$	$45.9^{b}\pm5.5$	$50.4^{b}\pm 6.5$	
Guar gum (T3)	1.5	$45.0^{bc} \pm 4.0$	$45.5^{bc} \pm 4.1$	$45.8^{bc} \pm 4.1$	$50.5^{bc} \pm 5.8$	
Reducing sugars (%)						
Xanthan gum (T1)	1.5	33.8 <sup>a</sup> ±2.2	35.8 <sup>a</sup> ±2.6	37.1 <sup>bc</sup> ±3.8	$40.0^{\circ}\pm4.6$	
Pectin (T2)	1.5	$28.6^{b}\pm2.8$	$35.8^{ab}\pm 2.6$	37.9 <sup>b</sup> ±2.8	$42.6^{b}\pm4.1$	
Guar gum (T3)	1.5	$32.5^{bc} \pm 2.8$	$38.5^{bc} \pm 2.8$	42.7 <sup>a</sup> ±3.9	47.1 <sup>a</sup> ±4.3	
<u>Total sugars (%)</u>						
Xanthan gum (T1)	1.5	$42.8^{a}\pm5.1$	$44.8^{b} \pm 4.1$	$47.8^{\rm c}\pm4.1$	$55.5^{b} \pm 5.5$	
Pectin (T2)	1.5	$37.5^{bc} \pm 2.8$	$45.2^{bc} \pm 4.2$	$47.9^{b}\pm4.2$	$54.4^{\circ}\pm5.6$	
Guar gum (T3)	1.5	40.3 <sup>b</sup> ±4.2	52.4 <sup>a</sup> ±4.4	55.7 <sup>a</sup> ±4.1	$65.5^{a}\pm 5.8$	

Data are expressed as mean  $\pm$  standard deviation; The values with different lower-case letters in each column are significantly (p  $\leq$  0.05) different from each other; T1: Sea buckthorn squash with xanthan gum; T2: Sea buckthorn squash with pectin; T3: Sea buckthorn squash with guar gum

		Storage period			
Hydrocolloid treatments	Conc. (%)	0 day	30 days	60 days	90 days
		Ascorbic	acid (mg 100 g <sup>-1</sup> )		
Xanthan gum (T1)	1.5	150.3 <sup>a</sup> ±10.1	145.3 <sup>b</sup> ±10.0	129.1°±7.3	$115.9^{\text{d}}\pm10.4$
Pectin (T2)	1.5	$150.2^{ab} \pm 10.2$	150.2a±10.0	$136.2^{a} \pm 7.4$	126.9 a± 10.5
Guar gum (T3)	1.5	$148.2^{c} \pm 10.2$	$144^{bc} \pm 10.2$	130.3 <sup>b</sup> ±7.5	$120.3^{\text{b}}\pm10.1$
Carotenoid content (mg 100 g <sup>-1</sup> )					
Xanthan gum (T1)	1.5	$72.0^{bc}\pm7.1$	$67.4^{a} \pm 6.1$	$61.2^{\mathrm{a}}\pm 6.2$	$44.0^{bc} \pm 4.5$
Pectin (T2)	1.5	$72.4^{b} \pm 6.1$	$66.4^{b} \pm 6.3$	$60.5^{\circ} \pm 6.1$	$44.8^{b} \pm 5.5$
Guar gum (T3)	1.5	$73.3^{a} \pm 6.2$	$65.3^{bc} \pm 6.4$	$61.2^{ab}\pm6.7$	$45.5^{a} \pm 5.1$

Table 3: Effect of hydrocolloids	and storage on	ascorbic acid	and carotenoid	content of sea
buckthorn squash				

Data are expressed as mean  $\pm$  standard deviation; The values with different lower-case letters in each column are significantly (p  $\leq$  0.05) different from each other; T1: Sea buckthorn squash with xanthan gum; T2: Sea buckthorn squash with pectin; T3: Sea buckthorn squash with guar gum

# Total carotenoids and ascorbic acid

Carotenoids are important food components due to their colour and nutritional value as provitamin A apart from being strong antioxidants. Significant (p<0.05) reduction in total carotenoids was found in sea buckthorn squash as compared to pulp (Table 1). A significant declining trend was also observed in squash during storage in all the treatments (Table 3). Similar trends of declining in carotenoid content of stored products were noticed by Verma and Sastry (1969) in orange squash and Krumreich *et al.* (2018) in guava nectar. In present study, the addition of 1.5% pectin exhibited higher carotenoid levels 44.8 mg 100 g<sup>-1</sup> in sea buckthorn squash, followed by guar gum and xanthan gum which showed 45.5 and 44.0 mg 100 g<sup>-1</sup> at the end of storage period.

Vitamin C is an important constituent of our nutrition due to its antioxidant property (Burdurlu *et al.*, 2006). Ascorbic acid in squash was significantly (p<0.05) reduced in all the treatments than pulp (Table 1). Vitamin C is sensitive to light, heat and temperature, so is susceptible to loss. A significant reduction in ascorbic acid content of sea buckthorn squash was found in all the treatments during storage (Table 3). This reduction during storage could be attributed to the oxidation of ascorbic acid to dehydro-ascorbic acid (Damame *et al.*, 2002). The incidence of light in transparent glass packaging and storage temperatures may also have caused decrease in vitamin C levels (Carvalho *et al.*, 2005). Barwal *et al.* (2002) reported decline in ascorbic acid in plum-seasoned squash during 6 months storage. However, in present study, at the end of storage the addition of 1.5% pectin in sea buckthorn squash caused high ascorbic acid content (126.9 mg 100 g<sup>-1</sup>) as compared to xanthan and guar gum (Table 3).

### Antioxidant activity and total phenolic content

DPPH method has broadly been used to test the ability of chemical compounds to act as free radical scavengers and to assess the antioxidant capacity of foods and plant extracts. Antioxidant activity and total phenolic content of sea buckthorn pulp and squash incorporated with three hydrocolloids *viz.*, pectin, guar gum and xanthan gum is presented in Table 1. Sea buckthorn squash had significantly low antioxidant activity as compared to pulp. Significant reduction in antioxidant activity was found in sea buckthorn squash during storage in all the treatments (Table 4). The reduction in antioxidant activity is because of the loss of phenolic compounds, carotenoids and vitamin C during storage, which was more severe in xanthan gum treatment (74.3-36.6%) than other treatments (85.4-76.6%) in pectin and (81.4-66.6%) in guar gum. Decrease in antioxidant activity during storage has previously been reported by Rababah *et al.* (2011a,b) in strawberry jam.

	_		Storage pe	riod	
Hydrocolloid treatments	Conc. (%)	0 days	30 days	60 days	90 days
	Antioxida	nt activity (% i	<u>nhibition)</u>		
Xanthan gum (T1)	1.5	$74.3^{c} \pm 6.2$	$54.1^{\circ} \pm 5.1$	$44.3^{c} \pm 4.1$	$36.6^{\circ} \pm 3.4$
Pectin (T2)	1.5	$85.4^{ab}\pm7.3$	$84.7^{a}\pm 6.2$	$84.2^{a}\pm7.2$	$76.6^{a} \pm 5.4$
Guar gum (T3)	1.5	$81.4^{a}\pm6.3$	$80.3^{b}\pm4.1$	$74.1^{b}\pm 6.2$	$66.6^b \pm 6.4$
Total phenolic content (mg GAE 100 g <sup>-1</sup> )					
Xanthan gum (T1)	1.5	$3.2^{bc} \pm 0.5$	$2.4^{c}\pm0.6$	$2.0^{b} \pm 0.6$	$1.4^{\mathrm{a}} \pm 0.1$
Pectin (T2)	1.5	$3.6^{ab}\pm0.2$	$3.2^a \pm 0.8$	$2.4^{a}\pm0.7$	$1.7^{ab}\pm0.2$
Guar gum (T3)	1.5	$3.4^{a}\pm0.1$	$2.9^{ab} \pm 0.9$	$2.0^{bc}\pm0.5$	$1.2^{c} \pm 0.2$

Table 4: Effect of hydrocolloids and storage on	antioxidant activity and total phenolic content
of sea buckthorn squash	

Data are expressed as mean  $\pm$  standard deviation; The values with different lower-case letters in each column are significantly (p  $\leq$  0.05) different from each other; T1: Sea buckthorn squash with xanthan gum; T2: Sea buckthorn squash with pectin; T3: Sea buckthorn squash with guar gum

A significant reduction in total phenolic content was observed in sea buckthorn squash than pulp (Table 1). The impact of hydrocolloids and storage on total phenolic content of squash is given in Table 4. Total phenolic contents showed significant decrease in sea buckthorn squash during storage in all the treatments. This could be due to the susceptibility of phenolic components to oxidation during storage (Selvamuthukumaran and Farhath, 2013). Reduction in total phenolic content was more severe in guar gum treatment (3.4-1.2 mg GAE 100 g<sup>-1</sup>), followed by xanthan gum (3.2-1.4 mg GAE 100 g<sup>-1</sup>) and pectin (3.6-1.7 mg GAE 100 g<sup>-1</sup>) during storage. A declining trend of total phenols in jamun squash has been reported by Kannan and Susheela (2004) during the period of 6 months of storage.

### Phenolics composition by HPLC

HPLC analysis of sea buckthorn samples showed the presence of three phenolic compounds, namely protocatechinic acid, quercetin and kaempferol. Of these, protocatechinic acid was the highest component in sea buckthorn pulp and squash. However, protocatechinic acid, quercetin and kaempferol during storage were significantly reduced in sea buckthorn squash in all the treatments as

		Storage period			
Hydrocolloids treatments	Conc. (%)	0 day	30 days	60 days	90 days
	Prot	ocatechinic aci	<u>d</u>		
Xanthan gum (T1)	1.5	$28.99^{ab} \pm 4.8$	26.99 <sup>b</sup> ±4.8	24.6 <sup>b</sup> ±2.2	$18.4^{b}\pm1.4$
Pectin (T2)	1.5	29.50 <sup>a</sup> ±3.5	$27.5^{a}\pm3.5$	$24.80^{a}\pm2.0$	$17.4^{c}\pm1.5$
Guar gum (T3)	1.5	$27.9^{\circ}\pm3.8$	25.9°±3.8	$22.6^{\circ} \pm 3.2$	19.3 <sup>a</sup> ±1.6
		Quercetin			
Xanthan gum (T1)	1.5	$11.5^{a} \pm 1.2$	$10.5^{a}\pm1.1$	$7.5^{ab} \pm 1.2$	$5.4^{a}\pm1.1$
Pectin (T2)	1.5	$11.4^{abc} \pm 1.3$	$8.5^{b}\pm1.5$	$7.7^{a} \pm 1.1$	$4.3^{b}\pm1.4$
Guar gum (T3)	1.5	$11.5^{ab} \pm 1.1$	$7.4^{c} \pm 1.6$	$5.5^{c} \pm 1.1$	3.3°±1.1
Kaempferol					
Xanthan gum (T1)	1.5	$7.4^{bc} \pm 1.3$	$4.8^{\circ}\pm1.8$	3.1 <sup>b</sup> ±0.06	$1.9^{a}\pm0.02$
Pectin (T2)	1.5	$7.8^{a} \pm 1.1$	$6.0^{a}\pm1.6$	$4.7^{a} \pm 1.0$	3.3 <sup>a</sup> ±0.01
Guar gum (T3)	1.5	$7.8^{ab} \pm 1.2$	$4.7^{b}\pm1.5$	$2.8^{bc} \pm 0.1$	$1.4^{c}\pm0.02$

able 5: Effect of hydrocolloids and storage on phenolic composition of sea buckthorn squash us	sing
HPLC (mg kg <sup>-1</sup> )	

Data are expressed as mean  $\pm$  standard deviation; The values with different lower-case letters in each column are significantly (p  $\leq$  0.05) different from each other; T1: Sea buckthorn squash with xanthan gum; T2: Sea buckthorn squash with pectin; T3: Sea buckthorn squash with guar gum

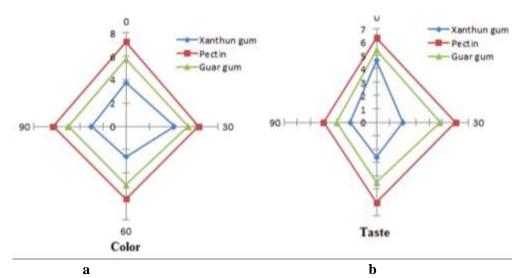


Fig. 2: Effect on sensory parameters of sea buckthorn squash (colour and taste)

compared to pulp (Table 1). Sea buckthorn squash during 90 days storage revealed that more phenolic compounds like protocatechinic acid, quercetin and kaempferol than in sea buckthorn pulp (Table 4). After processing, considerable decrease in these phenolic compounds were observed during storage in all the treatments. Decrease in phenolic components during storage may be due to the susceptibility of these compounds to oxidation (Rababah *et al.*, 2011b).

# Sensory evaluation

Sea buckthorn squash was subjected to sensory analysis after chemical analysis to assess its acceptance among consumers. Ascribed scores attributed to squash samples by trained taste panelists using 9-point hedonic scale (1 = dislike extremely; 9 = like extremely). Colour of any product is an important quality trait that directly reflects the extent of consumer preference and ultimate acceptance. Initially, sea buckthorn squash containing 1.5% pectin showed significantly higher colour value (7.2) and xanthan gum had lowest value (3.7) [Fig. 2a]. Taste value of squash containing 1.5% pectin also showed initially high score (6.5) followed by guar gum (5.3) and xanthan gum (4.8) [Fig. 2b]. However, the colour and taste decreased significantly during 90 days storage. Decrease in colour score with storage could be due to the non-enzymatic browning reaction and oxidation of phenolic compounds. Decrease in taste during storage could be due to oxidative reactions within the product.

**Conclusion:** The preparation of sea buckthorn squash is a viable alternative as noticed from the physicochemical, phenolic, antioxidant and sensory properties of product. These properties of sea buckthorn squash were affected by hydrocolloids addition. Increase in TSS, reducing and total sugars was observed in sea buckthorn squash than pulp. The sea buckthorn squash exhibited lower values of ascorbic acid, carotenoids, phenolics and thus exhibited lower antioxidant activities as compared to pulp. During storage, the squash containing pectin, in turn, showed higher stability of ascorbic acid and phenolic compounds, which directly influenced the greater antioxidant activity when compared to other treatments containing xanthan gum and guar gum. Hence, sea buckthorn berries have bio-industrial potential and advanced research is desired for further improvement and betterment of value-added products by utilizing the seasonal fruits like sea buckthorn.

**Acknowledgment:** The first author thankful to the Department of Biotechnology, Government of India, New Delhi for providing financial support to the present study.

Conflict of interest: All authors declare that there is no conflict of interest.

#### Ursila Irshad et al.

#### REFERENCES

- Abad-Garcia, B., Berrueta, L.A., Lopez-Marquez, D.M., Crespo-Ferrer, I., Gallo, B. and Vicente, F. 2007. Optimization and validation of a methodology based on solvent extraction and liquid chromatography for the simultaneous determination of several polyphenolic families in fruit juices. *Journal of Chromatography A*, **1154**: 87-96.
- Andersson, S.C., Olsson, M.E., Johansson, E. and Rumpunen, K. 2009. Carotenoids in sea buckthorn (*Hippophae rhamnoides* L.) berries during ripening and use of pheophytin as a maturity marker. *Journal of Agriculture and Food Chemistry*, 14: 250-258.
- AOAC. 2005. *Official Method of Analysis* (18th edn.). Association of Official Analytical Chemists, Maryland, USA.
- Barwal, V.S. and Shrera, S.K. 2009. Standardization of extraction methods and preservation techniques of hill lemon juice. *Journal of Scientific and Industrial Research*, **68**: 608-610.
- Barwal, V.S., Sharma, R. and Lal, B.B. 2002. Use of sorbitol for the preparation of plum seasoned squash. *Journal of Food Science and Technology*, **39**: 413-417.
- Brand-williams, W., Cuvelier, M.E. and Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology*, **28**: 245-251.
- Burdurlu, H.S., Koca, N. and Karadeniz, F. 2006. Degradation of vitamin C in citrus juice concentrates during storage. *Journal of Food Engineering*, **74**: 211-216.
- Carvalho, J.M., Maia, G.A., Figueiredo, R.W., Brito, E.S. and Garruti, D.S. 2005. Bebidamista com propriedadeestimulante à base de água de coco e suco de cajuclarificado. *Ciencia Tecnologia de Alimentos.*, **25**(4): 813-818.
- Damame, S.V., Gaikwad, R.S. and Patil, S.R. 2002. Vitamin C content of various amla products during storage. *Orissia Journal of Horticulture*, **30**: 19-22.
- Deka, B.C. and Sethi, V. 2001. Preparation of mixed fruit juice spiced ready to serve beverages. *Indian Food Packer*, **42**: 8-61.
- Durand, A., Franks, G.V. and Hosken, R.W. 2003. Particle sizes and stability of UHT bovine, cereal and grain milks. *Food Hydrocolloids*, **17**: 671-678.
- Eccleston, C., Baoru, Y., Tahvonen, R., Kallio, H., Rimbach, G.H. and Minihane, A.M. 2002. Effects of an antioxidant-rich juice (sea buckthorn) on risk factors for coronary heart disease in humans. *Journal of Nutritional Biochemistry*, **13**: 346-354.
- Hussain, I., Zeb, A., Shakir, I. and Shah, A.S. 2008. Combined effect of potassium sorbate and sodium benzoate on individual and blended juices of apricot and apple fruits grown in Azad Jammu and Kashmir. *Pakistan Journal of Nutrition*, **7**: 181-185.
- Iqbal, A., Yasmin, S., Wadud, S. and Shah, W.H. 2001. Production storage packing and quality evaluation of guava nectar. *Pakistan Journal of Food Science*, **11**: 33-36.
- Javanmard, M. and Endan, J. 2010. A survey on rheological properties of fruit jams. *International Journal of Chemical Engineering and Applications*, **1**(1): 31-37.
- Kannan, S. and Susheela, T.A. 2004. Studies on the storage life of jamun (*Syzygium cuminii* Rom.) fruit products. *Journal of Food Science and Technology*, **41**: 186-188.
- Krumreich, F.D., Correa, A.P.A., Nachtigal, J.C., Buss, G.L., Rutz, J.K., Crizel-Cardozo, M.M., Jansen, C. and Zambiazi, R.C. 2018. Stabilization of guava nectar with hydrocolloids and pectinases. *Polimeros*, 28(1): 53-60.
- Kumar, R., Kumar, G.P., Chaurasia, O.P. and Singh S.B. 2011. Phytochemical and pharmacological profile of sea buckthorn oil: A review. *Research Journal of Medicinal Plants*, **5**: 491-499.
- Labuza, T.P. 2011. Hydrocoloids in bakery fillings pp. 67-109. In: Hydrocolloids in Food Processing (eds. B.M. Nieto and M. Akins). Institute of Food Technologies, Blackwell Publishing Ltd. [https://doi.org/10.1002/9780813814490.ch5]

- Li, T.S.C. and Wang, L.C.H. 1998. Physiological components and health effects of ginseng, Echinacea and sea buckthorn. pp. 329-356. In: *Functional Foods, Biochemical & Processing Aspects* (Ed. G. Mazza) Technomic publishing Co., Lancaster, USA.
- Rababah, T.M., Al-Mahasneh, M.A., Kilani, I., Yang, W., Alhamad, M.N. and Ereifej, K. 2011a. Effect of jam processing and storage on total phenolics, antioxidant activity and anthocyanins of different fruits. *Journal of the Science of Food and Agriculture*, **91**: 1096-1102.
- Rababah, T.M., Al-udatt, M.H., Al-mahasneh, M.A., Feng, H., Abdulaziz, M., Alothman, A., Yang, W., Kilani, I., Alhamad, M.N., Ereifej, K. and Abu-Darwish, M. 2011b. Effect of storage on the physicochemical properties, total phenolic, anthocyanin and antioxidant capacity of strawberry jam. *Journal of Food Agriculture and Environment*, 9(2): 101-105.
- Ranganna, S. 1986. *Prospects of Fruit and Vegetable Products*. Tata McGraw Hill Publishing Co., New Delhi, India.
- Rousi, A. and Aulin, H. 1997. Ascorbic acid content in relation to ripeness in fruits of six *Hippophae rhamnoides* clones from Phyhaeranta, SW Finland. *Annales in Agriculturae Fenniae*, **16**: 80-87.
- Sadar, L. N. 2004. *Rheological and Textural Characteristics of Copolymerized Hydrocolloidal Solutions Containing Curdlan Gum.* MSc thesis University of Maryland, Maryland, USA.
- Saggu, S., Davekar, H.M., Gupta, V., Sawhney, R.C., Banerjee, P.K. and Kumar, R. 2007. Adaptogenic and safety evaluation of sea buckthorn (*Hippophae rhamnoides*) leaf extract: A dose dependent study. *Food and Chemical Toxicity*, 45: 609-617.
- Santos, V.E., Casas, J.A. and Go, E. 2000. Xanthan gum: Production, recovery and properties. *Biotechnology Advances*, **18**: 549-579.
- Selvamuthukumaran, M. and Farhath, K. 2013. Development of spiced sea buckthorn [Elaeagnus rhamnoides (L.) A. Nelson syn. Hippophae rhamnoides L.] mixed fruit squash. Indian Journal of Traditional Knowledge, 13: 132-141.
- Selvamuthukumaran, M., Khanum, F. and Bawa, S. A. 2007. Development of sea buckthorn mixed fruit jelly. *International Journal of Food Science and Technology*, **42**: 403-410.
- Sharma, P. and Gujral, H.S. 2010. Antioxidant and polyphenols oxidase activity of germinated barley and its milling fractions. *Food Chemistry*, **120**: 673-678.
- Shimoda, M. and Osajima, Y.1981. Studies on off-flavor formed during storage of *Satsuma mandarin* juice. *Journal of Agricultural Chemical Society of Japan*, **55**: 319-324.
- Shreshta, M.K. and Bhatia, B.S. 1982. Apple juice: Physicochemical characteristics and storage studies. *Indian Food Packer*, **36**: 53-60.
- Sogi, D.S. and Singh, S. 2001. Studies on bitterness development in kinnow juice, ready to serve beverage, squash, jam and candy. *Journal of Food Science and Technology*, **38**: 433-438.
- Swamy, J.S., Reddy A.H. and Banik, A.K. 2011. Stabilization of watermelon squash with xanthan gum and its organoleptic evaluation. *International Journal of Processing and Post-Harvest Technology*, **2**(1): 24-28.
- 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.