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IMPACT OF GEOGRAPHICAL VARIATIONS ON NUTRITIONAL QUALITY OF SEA BUCKTHORN IN NORTHERN PAKISTAN

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ABSTRACT

Sea buckthorn (*Hippophaė rhamnoides L.*), an ancient crop with modern virtues has recently gained worldwide attention, mainly for its nutritional and medicinal value. Nowadays, more attention has been given to medicinal plants and natural food sources due to their nutraceutical properties, presence of bioactive constituents and associated health claims. In this scenario, Sea buckthorn (*Hippophae rhamnoides L.*), has shown promising results as a natural curative agent for several diseases. The present project has been designed to evaluate the impact of different geographical locations of Northern Pakistan (Khaplu, Shigar, Gilgit and Ghizar) on the nutritional significance and antioxidant potential of sea buckthorn components (seed, berry and whole berry). The compositional analysis of sea buckthorn components revealed that the berry's dominant minerals were potassium, iron and sodium, while the seed's content of calcium magnesium and iron was higher. All the components of sea buckthorn also contained appreciable quantity of crude protein, crude fat and crude fiber. Similarly, the highest amount of vitamin C and A was witnessed in berries of Khaplu and Shighar origin, respectively. The fatty acid profile revealed the highest concentration of unsaturated fatty acids in the seed portion with the dominant presence of linoleic acid and γ-linolenic acid. Essential amino acids were reported maximum in the berry portion followed by whole berry powder. The assessment of antioxidant potential revealed maximum TPC in berries from Shighar, TFC in seeds from Khaplu and antioxidant potential in berries from Khaplu.

Keywords: Sea buckthorn, Unsaturated fatty acids, Essential amino acids, Fibers, Antioxidant potential

Introduction

Plant-based foods, especially fruits and vegetables are gaining popularity as a means of preventing and treating chronic diseases. In this scenario, sea buckthorn (*Hippophae rhamnoides*), an ancient crop with modern virtues has shown promising results as a natural curative agent against CVDs [1]. Naturally, sea buckthorn comes under the category of berry. It is a hardy bush that belongs to the family *Elaeagnaceae*. The cold and dry regions with 2500-4200m elevation (above the sea level) are idea habitat for the growth of sea buckthorn plant. Geographically, it can be found in both Asia (particularly Pakistan, India, and China) and Europe (Russia, Germany, Finland, Sweden, and Norway) [2]. Number of *Hippophae* species is still a controversial topic; however, seven species

have been well documented. *Hippophae tibetana, Hippophae rhamnoides,* and *Hippophae salicifolia* majorly exist in Indo-Asian regions. While *Hippophae rhamnoides* L. ssp. *Turkestanica* is found mainly in Pakistan [3].

It is locally known as *Buru* and is widely distributed in hilly areas of Pakistan including Skardu, Gilgit-Baltistan, Swat, Chitral, Kurram Agency and Ladak. Besides native growth, it is also now cultivated by the farmers. In 2007, it was cultivated in an area of 4,000 hectares in Pakistan [4]. In Northern regions of Pakistan, its ripened season is September and November while it may be collected from the market until January. Despite its abundance and timely ripening, it is rarely exploited for diverse usages and is only marginally processed for value addition as juice, jam, squash, and oil [5]. Sea buckthorn has been domesticated all over the world because of its nutraceutical properties. Chinese declared it their traditional medicine thousand years ago. Recently, the scientific community has noticed this berry plant due to its remarkable nutritional profile and therapeutic potential. Sea buckthorn barriers are composed of pulp (67%), seed (24%) and peel (9%). Nutritionally, the most important components are seeds and pulp. These berries are packed with carbohydrates, protein, dietary fibers, vitamins (A, C, E, B_1, B_2, B_3, B_6, lycopene and β-carotene), 22 fatty acids including omega fatty acids (omega 3, 6, 9 and 7), polyunsaturated fatty acids (Oleic acid, palmitoleic acid and vaccenic acid), 20 mineral elements (Ca, Mg, Fe, Zn, Cr, Cu, K and Se), polyphenols (epigallocatechin, epicatechin, gallic acid, proanthocyanidins, chlorogenic acid) phytosterols and flavonoids [6].

Despite its high nutritional value, fresh barriers are rarely eaten because of their strong acidic taste. However, its juice can be mixed with other foods as well as dehydrated for the diverse nature of food applications [7]. The levels of phytochemicals in SBT berry tend to vary according to climatic variations, the origin of the berry and techniques used for their extraction. Different types of flavonoids including apigenin, quercetin, myricetin, kaempferol and luteolin are abundantly present in the pulp portion. SBT seed oil is a good source of lanosterol, oleanol aldehyde, campesterol, cycloeucalenol, α-amyrin, clerosterol sitosterol, avenasterol, β-amyrin, sitostanol, avenasterol, stigmasta-en-ol, stigmastadienol, methylenecycloartanol,lupeol, gramisterol, sitosterol,cycloartenol, methylobtusifoliol, erythrodiol, citrostadienol and uvaol [8]. Despite this, current study aimed to compare the nutritional and bioactive potential of sea buckthorn from various regions of Pakistan because there are so few scientific studies examining this fruit and its possible applications.

Material and Methods

Procurement and Preparation of Raw Materials

Sea buckthorn whole berries were procured from different locations in Northern areas of Pakistan including Shigar (Sh), Khaplu (Kh), Ghizer (Gh) and Gilgit (Gi). The reagents required for analysis were purchased from Merck (Germany) and Sigma-Aldrich (USA).

Preparation of Raw Materials

Sea buckthorn berries were washed to remove dirt, dust, twigs or any other impurities followed by dehydration at 60°C in lab scale dehydrator (Harvest Saver R-5A, Commercial Dehydrator Inc., USA). Afterward, whole berries, their seeds and the berry portion of the respective sample were converted into powder, packed in air-tight glass jars and stored at room temperature (25°C) for further use in the study. Subsequently, crude oil was extracted from both sea buckthorn seeds and berry powder through solvent extraction using food-grade hexane [9].

Analysis of Sea Buckthorn Powder (SBP)

Proximate composition

The samples of sea buckthorn seeds, berries and whole berries were analyzed for proximate composition. The moisture content was determined by the oven drying method, crude fat was determined by the Soxhlet method and crude protein was assessed by the Kjedhal method. Whereas the total ash and nitrogen free extract (NFE) were determined by following the procedures stated in AACC(2010) [10].

Mineral contents

Samples were assessed for minerals including Na, K, Ca, Mg, Fe and Zn through Flame Photometer (Sherwood, Flame Photometer 410, Sherwood Scientific Ltd., Cambridge, UK) and Atomic Absorption Spectrophotometer (Varian AA240, Varian Medical Systems, Australasia Ltd., Belrose, Australia) using method described in AOAC(2019) [11]. The dried sample (0.5g) was digested on a hot plate (60-70°C) for 20 minutes using HNO_3 (10mL) and $HClO_4$ (5mL) at 190°C until the clear contents were obtained. Afterward, the sample was diluted in a volumetric flask (100mL) with double distilled water. The standard curve of each element was determined to find out the mineral contents in samples.

Vitamins

The vitamin A (total carotenoids) and C contents of raw material were determined by following their respective methods as described by Corbu *et al.* (2020) [12] and Esch *et al.* (2010) [13]. Total carotenoid contents in sea buckthorn seed and berry oil were determined through a spectrophotometer (Varian Cary 50 UV spectrophotometer, Varian Co., USA). The oil sample (1g) was mixed in n-hexane (50mL) and assessed for the concentration of total carotenoids at 450 nm absorbance. A calibration curve of β-carotene standard solution in n-hexane (0.1-7.0mg L^{-1}) was used to determine the carotenoid content (mg β-carotene/100g of oil) of samples.

The Vitamin C was determined by dissolving 2,6-dichloroindophenol (0.25g) in distilled water along with 500mL sodium bicarbonate (0.21g). The resulting solution was further diluted to 1L with distilled water. For ascorbic acid standardization, standard ascorbic acid solution (5 mL) was carefully pipetted in an Erlenmeyer Flask (250 mL). The concentration of the standard Vitamin C was recorded. For estimation of Vitamin C in the sample, DCIP solution filled in burette (50mL) was added drop by drop until a light red or pink color persistent for 30sec appeared. The volume of DCIP used to oxidize all the ascorbic acid was recorded. The procedure was repeated with two additional samples of standard vitamin C. By using the balance equation, the concentration of vitamin A (mg/L) was determined.

Fatty acid Profile

The fatty acid profile of berry and seed oil was determined by using a Gas Chromatograph (7890B, Agilent Technologies, Lake Forest, USA) following the method of Ranjith *et al.*, (2006)[14] methyl esters were prepared by the saponification of glyceride using NaOH and methanol. GC equipped with a capillary column (Rtx®-2560 biscyanopropyl polysiloxane, 100m long with a diameter of 0.25mm and the thickness of the stationary phase 0.20µ), a split-less injector (temperature 225°C) and a flame ionization detector (temperature 250°C) was used with a sample volume of 1µL. Start column temperature was 100°C with holding time of 4min. The oven temperature was increased at the rate of 3°C/min to 240°C/min, holding for 11min. Nitrogen as carrier gas was used (99.99%) at a constant flow rate of 1.2 mL/min. The hydrogen flow was 30mL/min, the airflow was 250mL/min, and the makeup gas flow (nitrogen) was 45mL/min. FA's in samples were identified by comparison with retention times of 37 FA methyl ester standards at the same conditions. The results were expressed as a percentage (%) of individual fatty acids to total fatty acids.

Amino acid profile

An amino acid analyzer (Hitachi L8500, Tokyo, Japan) was used for the estimation of essential- and non-essential amino acids in the SBT berry, seed and whole berry samples using the method of Walsh and Brown (2000) [15]. Defatted samples (30mg) were taken in glass ampoules along with a 30mg powdered sample, 5mL of 6M HCl and 5 µmol norleucine. Then liquid nitrogen was used to evacuate ampoules. Afterward, ampoules were packed and hydrolyzed for 24 hrs at 110°C in an oven. Then ampoules were cooled, and their tips were broken. The filtrates were dried at 40°C under a vacuum in a rotary evaporator. The residues were then dissolved in 5µL (for neutral and acidic amino acids) or 10µL (for basic amino acids) acetate buffer having pH 2.2 and the solutions were dispensed into the container of the amino acid analyzer. The peak area of individual amino acids in the sample was matched to the standard amino acid protein hydrolysate to perform quantification.

Antioxidant potential

Total phenolic content (TPC)

The anti-oxidant assay was carried out by taking a sample (0.3g) in amber glass vials (40 mL). After adding methanol (100 %; 15 mL), sonication was carried out thrice for 15min with intervals of 10min maintaining the temperature below 30ºC. The crude extract was centrifuged at 3000g for 15 minutes. The sample in vials was kept at 76 ºC until analysis. Subsequently, the gallic acid standard of sample extract (20µL) was gently mixed with 100mL of 0.2N the Folin-Ciocalteu's phenol reagent. About 80 mL of 7.5% (w/v) sodium carbonate was added and mixed to each well after 6min. The mixture was incubated at ambient temperature for 2hrs followed by measuring the absorption at 760nm using a FLUO star OPTIMA plate reader (BMG Labtech, Durham, NC, USA). The results were reported as mg of gallic acid equivalent (GAE) per g dry weight (mg GAE/g DW) [16].

Total flavonoid content (TFC)

A mixture of 1M aqueous potassium acetate and 0.1M aluminum nitrate (10%) was prepared in a test tube. After adding 0.5mL methanolic extract to the sample, the absorbance was measured at 415nm. The results were reported as mg of quercetin equivalent (QE) per g dry weight (mg QE/g DW) [17].

ABTS free radical scavenging activity

The ABTS cation radical solution (ABTS+) was prepared by using 5mL of 7mM ABTS solution and 88µL of 145mM potassium persulfate solution. The mixture was kept at room temperature, in the dark for 16 hours. After dilution with 80% ethanol, the absorbance was taken at 734nm. ABTS+ solution (12mL) was vigorously mixed with 120µL of sample extract. After 6min, the absorbance was taken at 734nm using ethyl alcohol as blank. The calibration curve was constructed using standard solutions of Trolox (100-2000µMTrolox/100g) in ethanol and the results were expressed in mM Trolox per 100g of sample [18].

Statistical analysis

Data associated with different attributes of the compositional analysis was evaluated by applying completely randomized design (CRD) through one and two-way ANOVA technique as explained by [19] along with repeated measures. The means were compared for the significant difference using the Tukey Test.

Results and Discussion

Analysis of Raw Materials

Mineral profile

Mean squares for selected minerals in seed, berry and whole berry exhibited significant difference concerning locations, treatments and their interaction. Calcium (83.65±0.39 mg/100g) and magnesium (71.61±0.40mg/100g) were abundantly present in seeds of Gilgit and Ghizar origin whereas the concentration of sodium (4.25±0.24 mg/100g) and zinc (5.47±0.23 mg/100g) was the least (Fig. 1a). The current findings revealed that the berry portion is also a good food source of essential minerals. Potassium (601.48±1.88 mg/100g) and iron (187.68±0.79mg/100g) were the most abundant minerals reported in berries while magnesium (26.39±0.71mg/100g) and zinc (1.21±0.07mg/100g) were minimum (Fig. 1b). Utilization of iron content is higher due to the presence of vitamin C content in abundance which facilitates its absorption. Moreover, SBT berry (an indigenous food) may help in the recovery of body minerals which are excreted frequently at high altitudes. It is evident from the results that the mineral profile of the whole berry was superior due to the contribution of minerals both from seed and berry portions (Fig 1c). The better proportion of mineral in whole berry makes it a better choice for supplementation in conventional foods.

The mineral content tends to vary significantly with the change in geographical location. These variations might be due to differences in genetic makeup, geographical location, fertility of

cultivated land, contamination of soil, agronomic practices and environmental conditions.

Prviously, Zeb (2004) reported greater variations in the contents of magnesium, calcium, iron and zinc in sea buckthorn seed samples [20]. In another study, the biochemical characterization of sea buckthorn seed from Pakistan was carried out by Zeb and Malook (2009). The mineral composition revealed the presence of sodium (47.65mg/100g), magnesium (75.8mg/100g), calcium (91.2mg/100g), zinc (9.65mg/100g), iron (29.02mg/100g) and potassium (8.8mg/100g) [21]. Similarly, Dhyani and his colleagues (2007) explored the basic nutritional attributes of SBT from different geographical locations in Uttarakhand Himalaya, India. The mineral assay exhibited the presence of iron (0.36-0.64mg/100g), magnesium (1.8-3.0mg/100g), zinc (0.49-2.8mg/100g), sodium (0.05-0.49mg/100g) and potassium (9.33-13.42mg/100g) [22].In a previous study Jaroszewska and his colleagues (2019), investigated the effect of varieties and mycorrhization on the chemical composition and antioxidants in SBT berries. The mineral profile showed the presence of iron (2.14±0.6mg/100g), magnesium (0.103±0.001mg/100g), zinc (2.63±.32mg/100g), sodium (0.09±0.001mg/100g) and potassium (1.82±.02 mg/100g) [23]. In a local study conducted by Sabir *et al* (2005), the berries collected from various locations of Gilgit- Baltistan were compared for the mineral profile. A wide variation was observed regarding potassium (25.9±0.75mg/100g), sodium (4.78±0.19mg/100g), calcium (9.98±0.29mg/100g), magnesium (19.8±0.71mg/100g), iron (13.3±0.43mg/100g) and phosphorus (12.3±0.11mg/100g) [24]. In another study, the presence of iron (321.4mg/100g), calcium (311.93mg/100g), magnesium (222.2mg/100g) and zinc (3.04mg/100g) were documented in dried berries of Chinese origin [18]. The findings of Stobdan *et al*., (2013) revealed that the concentrations of calcium, sodium, iron, zinc and manganese in SBT were many times higher than in commonly consumed fruits *i.e.,* peach, mango, banana apricot and orange [7].

Fig 1.a Mineral contents (mg/100g) of SBT seed portion of different locations

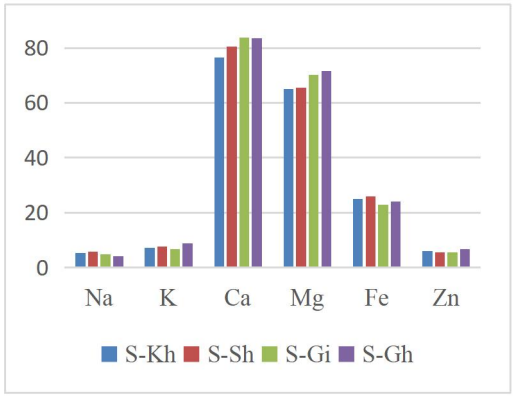


Fig 1.b Mineral contents (mg/100g) of SBT berry portion of different locations

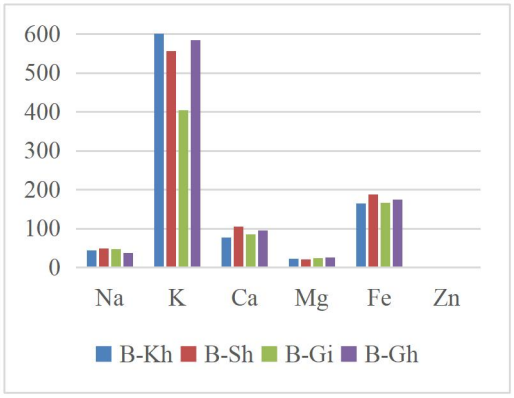
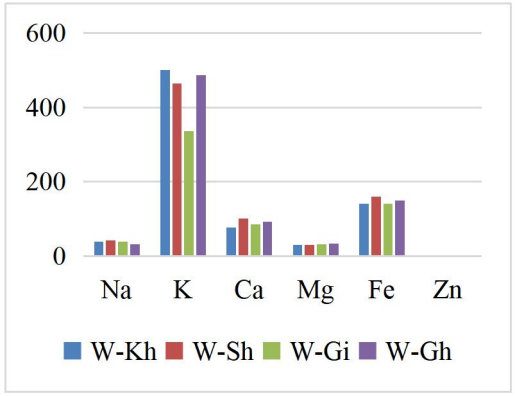


Fig 1.c Mineral contents (mg/100g) of SBT berry portion of different locations



Vitamins

Mean squares for vitamins of seed, berry and whole berry exhibited significant difference concerning concentrations of vitamin C and A. Furthermore, the interaction of locations and components was also significant. The vitamin C content of seeds were ranged form 33.66±0.85mg/100g (S-Gi) to 52.81±1.25mg/100g (S-Kh) (Fig.2a). Among different components of the maximum value for total carotenoids was observed in B-Sh (516.95±5.31) whereas the minimum was in S-Gi (28.47±1.18mg/100g) (Fig. 2b). The highest vitamin A content among all components were observed in W-Kh (227.02mg/100g) (Fig 2c). A limited experimental data is available for the elemental composition of sea buckthorn seed. The variations in the Vitamin C content of different locations indicated the biological diversity in SBT seed composition in Northern areas of Pakistan. In a previous study, George and Cenkowski (2005) evaluated the influence of harvest time on the quality of SBT seed and berry and reported the presence of 24.4±1.6 to 27.6±3.5 mg/100g carotenoids in seed oil with a non-significant increase in their concentration from September to January[25]. The findings of the current study are in line with those reported by Zeb and Malook (2009) . They explored the elemental composition of SBT seeds and reported 35.40±0.042mg/100g vitamin C content [21]. The variation in vitamin C content might be due to post-harvest processing and drying as vitamin C is labile to heat. Whereas, the high variation in carotenoids might be due to differences in genetic makeup, species, origin, drying techniques environmental conditions and altitude.

The current findings endorsed the superiority of SBT regarding vitamin C content; reported to be 10 times higher than kiwi (a commonly known rich source of ascorbic acid). Several studies have reported the concentration of vitamin C in SBT berries varied from 200 to 4000mg/100g depending upon species and verities [26]. The abundance of vitamin C in sea buckthorn berry with cardio-protective potential makes it a superior choice as a functional food. In a study, Gao and his co scientists explored the nutritional composition of SBT berries of European and Chinese origin and reported 28 to 310mg/100g and 200 to 2500 mg/100g of vitamin C content, respectively [27]. In another study, Buya *et al*., (2017) determined the functional components of dried sea buckthorn and found that European sea buckthorn berries contain 320mg/100g of vitamin C content [28]. Similarly, Nawaz and his colleagues (2019) documented the losses of vitamin C content in SBT berry during drying process (traditional and shade drying). The finding of the study revealed that the concentrations of vitamin C in dried berries varied between 6 to 275 mg/100g [29].

George and Cenkowski (2005) investigated the total carotenoids of SBT berry oil during different ripening stages and found carotenoid content ranged from 498.1±24.4 to 817.8±25.6mg/100g during the production (September to January) [25]. In another study, Ranjith *et al*., (2006) explored the composition of three species of SBT (*Hippophae rhamnoides, H. salicifolia, and H. tibetanacollected*) collected from different regions of the Indian Himalayas and found a significant variation in total carotenoid content of all species ranged from 64 to 342mg/100g [14]. Similarly, Buya *et al*., (2012) worked on the functional components of dried SBT berries and reported to contain 715.25±5.50mg/100g total carotenoid contents [28]. In an earlier study, Khan *et al*., (2005) documented the physicochemical aspects of sea buckthorn seed, pulp and whole berry collected from different locations in Northern areas of Pakistan including Sakrdu, Shigar, Khaplu and Hunza. The results showed that the whole berry from Khaplu had higher vitamin C content (35.54mg/100g) followed by Shigar (28.75mg/100g), Hunza (22.2mg/100g) and Sakrdu (21.23mg/100g) . Among the different components, the highest average ascorbic acid content was reported in pulp (38.12mg/100g) followed by whole berry (26.88mg/100) and seed (7.29mg/100g) [30].

Fig. 2a. Vitamin contents (mg/100g) of SBT seed portion of different locations

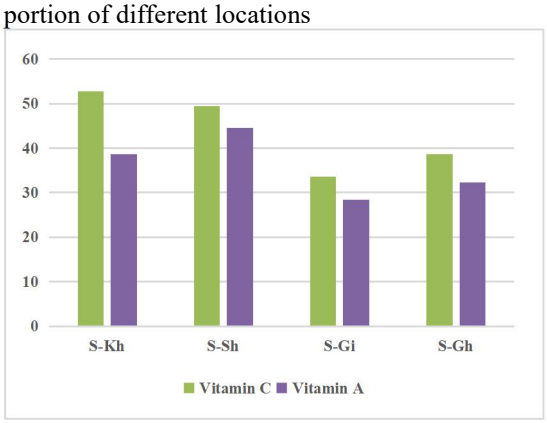


Fig. 2c. Vitamin contents (mg/100g) of SBT whole berry portion of different locations

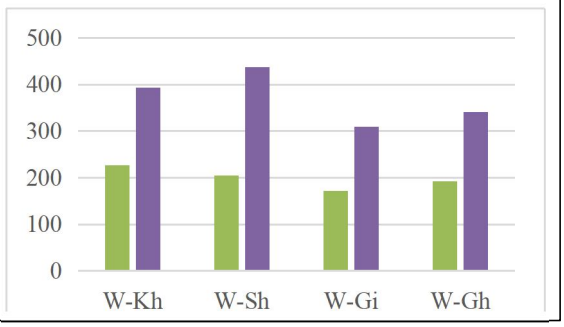
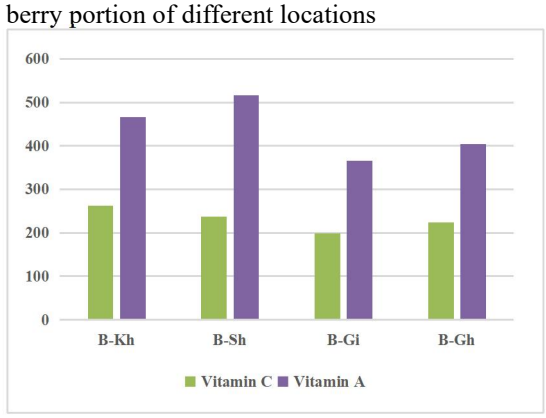


Fig. 2b. Vitamin contents (mg/100g) of SBT berry portion of different locations



Fatty acids

Mean squares for saturated and unsaturated fatty acids exhibited significant differences concerning seed, berry and whole berry. With reference to geographical locations, all fatty acids were significantly different except for pentadecanoic acid, arachidic acid and behenic acid. Furthermore, the interaction of locations with components was significant for palmitic acid, stearic acid, palmitoleic acid, oleic acid, vaccenic acid, linoleic acid and γ -linolenic acid. The overall concentration of saturated fatty acids in oil of different components was reported highest in B-Gi followed by S-Gh, S-Sh and S-Kh (Fig. 3a, 3b and 3c). Similarly, among unsaturated fatty acids content palmitoletic acid content was highest in in B-Kh whereas lowest in S-Kh (Fig. 3e, 3f, 3g). The maximum content of oleic acid was observed in B-Kh whereas the lowest was in S-Gh. The maximum content of linoleic acid was found in S-Kh whereas the lowest was in B-Kh. The maximum concentration of MUFAs and PUFAs was seen in W-Kh and W-Gi whereas the lowest was in W-Gh. The ratio of n-6 to n-3 was maximum in W-Gi followed by B-Kh whereas the lowest was in B-Gh followed by B-Sh. The berry oil of different cultivars had saturated fatty acids (42.00±2.07-45.54±2.77g/100g), and unsaturated fatty acids (49.54±2.05-55.68±2.57g/100g) with a wide range of n–6/n–3 (3.06-9.55). In contrast to the berry portion, the seed oil had higher amounts of USFAs (63.51±2.74-70.06±3.05g/100g) and lower amount of saturated fatty acids (29.84±2.30- 35.56±2.58g/100g) whereas, n–6/n–3 ranged from 1.38 to 1.49. Significant variations were observed between n-6/n-3 ratios of seed, berry and whole berry's oil. Various studies suggest high variation in the fatty acid profile of different components. Abid *et al*., (2007) reported that seed (14±0.21g/100g) and berry oil (36g/100g) contain saturated fatty acids whereas USFA's were 85±3.20 and 63.4±2.75g/100g, respectively[31].

Fig 3a. Saturated fatty acids (mg/100g) of SBT seed from different locations

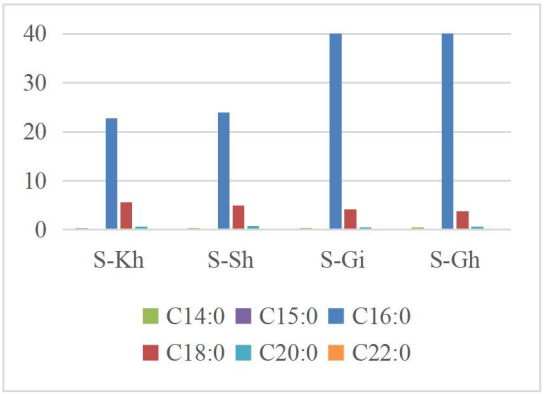


Fig 3b. Saturated fatty acids (mg/100g) of SBT berry from different locations

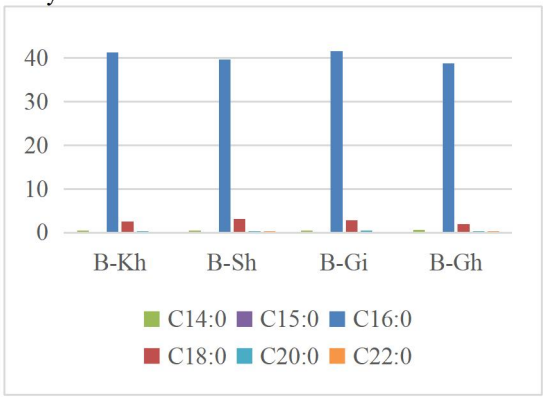


Fig 3c. Saturated fatty acids (mg/100g) of SBT whole berry from different locations

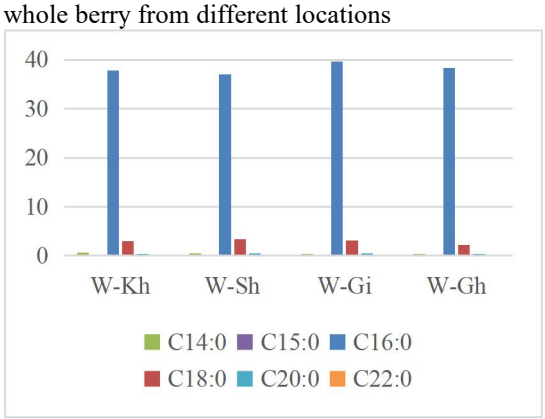


Fig 3d. Unsaturated fatty acids (mg/100g) of SBT seed from different locations

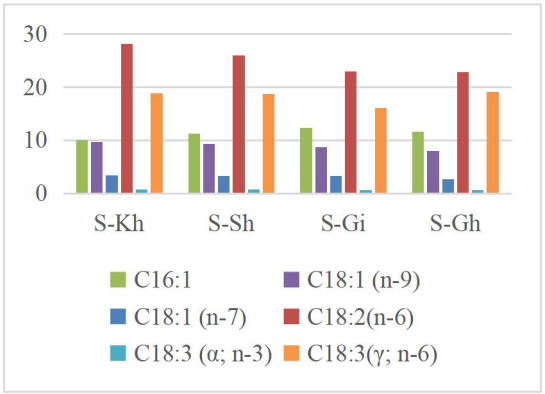


Fig 3e. Unsaturated fatty acids (mg/100g) of SBT berry from different locations

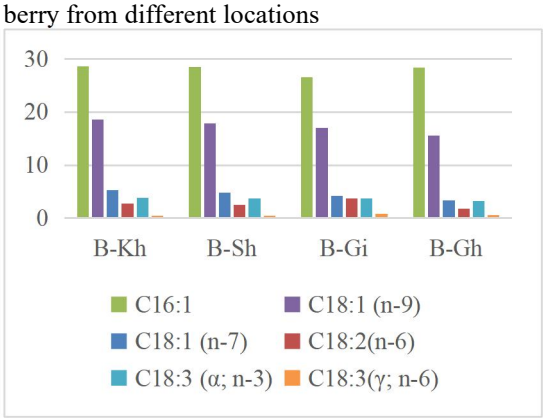
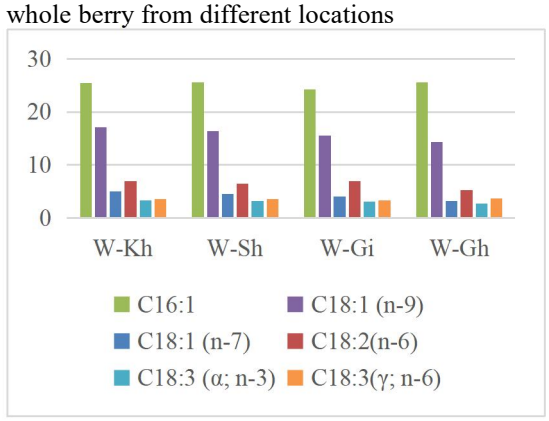


Fig 3f. Unsaturated fatty acids (mg/100g) of SBT whole berry from different locations



In an earlier study, Shafi *et al.*, (2008) investigated the variations in the fatty acid composition of sea buckthorn berry oil from 8 different locations in Northern areas (Pakistan). Palmitic acid (36.52 %) and palmitoleic acid (32.4%) were the major fatty acids along with oleic acid (37.07%), linoleic acid (12.36%) and linolenic acid (0.73%) [32].

The composition of berry oil revealed the concentration of unsaturated fatty acids was significantly higher than that of saturated fatty acids. The means for berry oil composition showed that the saturated fatty acids were the highest in B-Gi (45.54±2.77g/100g) whereas the lowest in B-Gh (42.00±2.07g/100g). The unsaturated fatty acids varied from 55.94±2.13 to 49.54±2.05g/100g in B-Gi and B-Gh. The maximum concentration of MUFAs and PUFAs was seen in B-Kh (52.41±1.78g/100g) and B-Gi 8.13±0.42g/100g) whereas the lowest was in S-Gh (47.23±1.32g/100g) and B-Gh (2.32±0.15g/100g), respectively. The ratio of n-6 to n-3 was maximum in B-Gi (9.55) followed by B-Kh (5.26) whereas the lowest (3.06) in (B-Gh).

An earlier investigation, Dulf, 2012) documented the fatty acid composition (seed, pulp/peel and whole berry) of sea buckthorn from different locations in Romania. The fatty acid profile of seed showed the oleic acid (13.57-16.74g/100g), linoleic acid (34.41 to 42.35g/100g) and α-linolenic acid (28.50-32.60 g/100g) as the most prominent fatty acids whereas palmitic acid (23-40%), oleic acid (20-53%) and palmitoleic acid (11-27%) were more concentrated in in berry portion [33].

Amino Acids

Mean squares for essential and non-essential amino acids exhibited significant differences concerning seed, berry and whole berry with respect to components (seed, berry and whole berry) and locations (Kh, Sh, Gi and Gh). The interaction between locations and components of SBT for essential and non-essential amino acids was significant. The mean values for essential amino showed the highest isoleucine content in S-Sh whereas the lowest was in B-Sh (Fig. 4a). The maximum concentration of leucine was found in B-Gh whereas lowest was in B-Sh. The lysine content was ranged from 0.38±0.08 to 2.99±0.15g/100g. The mean values for methionine showed maximum value in W-Gh whereas the lowest was in S-Gi. The findings for the phenylalanine depicted the highest value in S-Gi whereas the lowest in B-Gh. Similarly, the mean values of threonine varied from 2.94±0.25 to 10.40±0.65g/100g with highest value in S-Kh. The tryptophan was the highest in B-Sh whereas the minimum value was found in B-Gi. The contents of valine and histidine ranged from 3.02±0.12 to 20.28±1.29g/100g and 0.55±0.14 to 4.54±0.30g/100g, respectively. Among all components of different locations, the overall concentration of essential amino acids was found to be highest in B-Gi and lowest in W-Kh. Among different components of berries, non-essential amino acids were highest in whole berries. Among different non-essential amino acids, glutamine and cysteine was abundantly present in the seed and berry portions of Gilgat and Shighar respectively (Fig. 4b).

Fig. 4a. Essential amino acids of different components of sea buckthorn

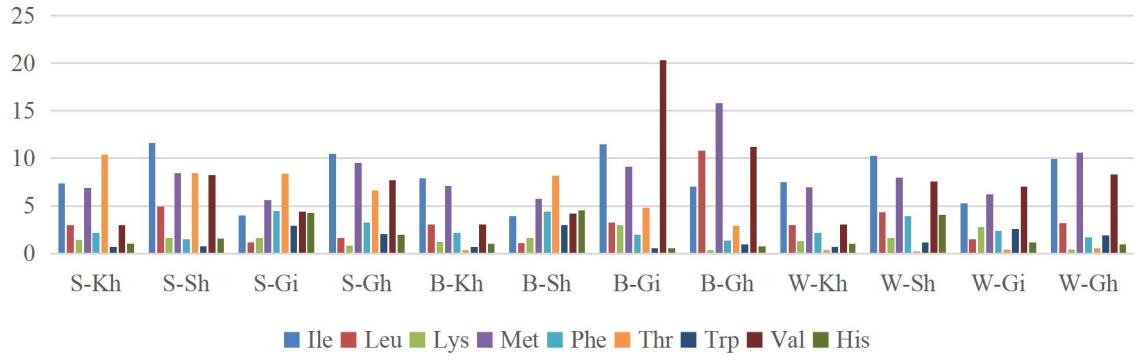
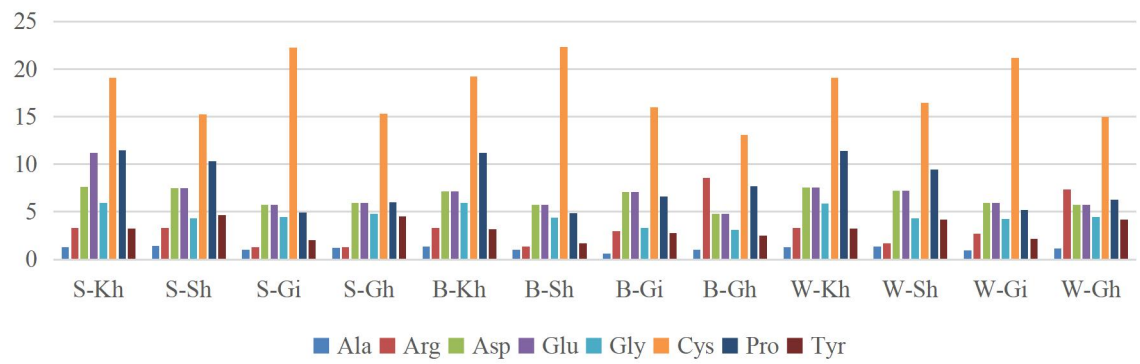


Fig. 4b. Non essential amino acids of diiferent components of sea buckthorn



In a previous study, Uransanaa *et al*., (2003) explored the protein and amino acid composition of seed buckthorn seeds. The findings revealed berry seeds as a major source of valuable protein (37.79%) with a sufficient amount of essential amino acids (43.32-45.04%). The compositional analysis of protein showed the concentration of essential amino acids; lysine (4.38g/100g), histidine (2.47g/100g), arginine (15.12g/100g), threonine (2.43 g/100g), valine (4.77g/100g), methionine (0.9g/100g), isoleucine (4.01g/100g), leucine (6.9g/100g) and phenylalanine (2.74g/100g) whereas the concentration of non-essential amino acids was cysteine (0.91g/100g), aspartic acid (10.92g/100g), serine (4.86g/100g), glutamic acid (22.51g/100g), proline (5.87g/100g), glycine (3.86g/100g), alanine (3.87g/100g) and tyrosine (2.47g/100g) [34]. The findings of current study regarding the overall concentration of essential and non-essential amino acids showed variation between different locations might be due to agronomic practices, climatic change and varietal differences [35].

The overall concentration of essential amino acids in the berry portion collected from different locations was found to be highest in B-Gi (55.08±3.26g/100g) followed by B-Gh (51.36±2.91g/100g) while the lowest was in B-Kh (36.54±2.33g/100g) and B-Sh (36.84±2.28g/100g). Whereas, the overall concentration of non-essential amino acids was highest in B-Sh (62.80±3.73g/100g) followed by B-Kh (62.55±3.80g/100g). The average essential amino acid content was comparatively higher in berry (44.95±2.69g/100g) than in seed (41.09±2.86g/100g), however, SBT seed had higher average non-essential amino acid content (58.81±3.31g/100g) compared to berry (54.82±3.11g/100g). The overall content of essential amino acids in the whole berry portion was found to be highest in W-Sh (49.19±3.31g/100g) followed by W-Gh (40.63±2.91g/100g), W-Gi (34.35±2.g/100g) and W-Kh (36.54±2.33g/100g). In a study, Ghimire and Sharma (2018) explored the nutritional aspects of underutilized species of sea buckhorn belonging to Hamalian Mountain. The findings raveled that SBT berry contained different concentrations of threonine (6.24mg/100g), serine (5.31mg/100g), glutamic acid (2.65mg/100g), glycine (0.64mg/100g), alanine (2.50mg/100g), cysteine (0.82 mg/100g), valine (2.85mg/100g), methionine (1.12mg/100g), tryptophan (0.51mg/100g), isoleucine (0.97mg/100g), leucine (1.94mg/100g), tyrosine (1.79mg/100g), phenylalanine (3.21mg/100g), histidine (1.06mg/100g), lysine (3.49mg/100g), arginine (0.47mg/100g) and proline (12.28mg/100g) [36].

Antioxidant potential

The mean values for total phenolic content (TPC) of sea buckthorn seed from different areas showed maximum TPC in S-Kh followed by S-Gh whereas the minimum was in S-Gi followed by S-Sh (Fig 5a, 5b, 5c). The total flavonoid content (TFC) of SBT seeds ranged from 15.69±1.74 to 30.52±.2.63mgQE/100g. Similarly, the ABST radical scavenging activity was maximum in S-Sh followed by S-Kh whereas the lowest values were in S-Gi followed by S-Gh.

Dolkar and his colleagues assessed the variability of bioactive compounds including antioxidants, phenolics and flavonoids in sea buckthorn seed of Himalayan origin. For this purpose, the berry samples were collected from nine different locations in the Himalayan mountains (Phey, Cholglamsar, Shey, Stakna, Chuchor, Dihar, Stakmo, Pheyang and Horzey) with altitudes ranging

from 3154±9 to 3794±15m. The findings illustrated the average phenolic content (108.3±33.2mgGAE/g DW) in seeds from different areas with 6.5 fold variation. However, the average total flavonoid content of different locations was 21.4±.6.2 mgQE/g DW of seed with 4.7 fold variation [16].

The findings of the current study comply with those reported by Korekar *et al.*, (2014), who investigated the variability in antioxidant properties and chemical composition of 17 populations of sea buckthorn belonging to different altitudes of the Himalaya ranging from 3214 to 3337m. The results revealed many folds variation among different populations regarding TPC (1-11), ferric reducing activity (1-8), free radical scavenging activity (1-14), ascorbic acid content (1-70) and carotenoids content (1-206). Moreover, maximum ascorbic acid content was reported in berries belonging to highest altitude [37].

Fig. 5a. Antioxidant potential of SBT seed portion from different locations

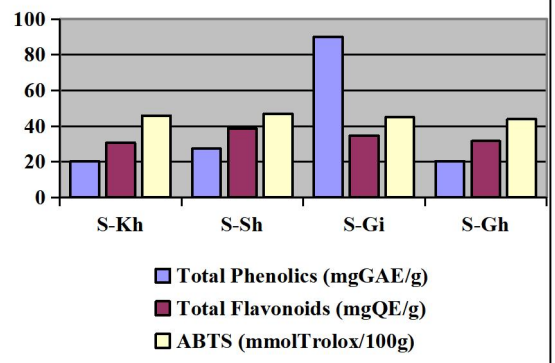


Fig. 5b. Antioxidant potential of SBT berry portion from different locations

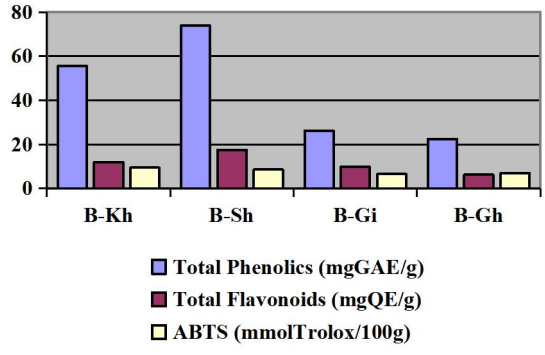
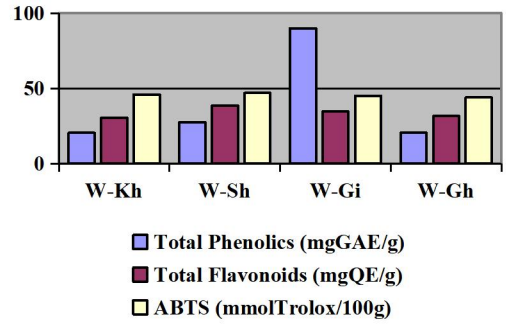


Fig. 5c. Antioxidant potential of SBT whole berry from different locations



Conclusion

Sea buckthorn (*Hippophae rhamnoides*), has shown promising results as a natural curative agent against several diseases. Recently, it has gotten the attention of the scientific community, because of its unique nutritional profile and therapeutic potential. Nutritionally, the most important components are pomace and seed which are packed with carbohydrates, protein, dietary fibers, vitamins, polyunsaturated fatty acids, mineral elements, polyphenols, phytosterols and flavonoids. The strong acidic flavor restricts its food applications which may be overcome by dehydration and partial incorporation of fruit in commonly used food products.

The mineral profile of all components of SBT revealed the presence of sodium (37.43±0.85 to 48.87±1.78mg/100g), potassium (556.39±1.60 to 601.48±1.88mg/100g), calcium (76.89±0.84 to 105.1±1.02mg/100g), iron (164.69±1.10 to 187.68±0.79mg/100g), magnesium (64.95±0.54 to 71.61±0.40mg/100g) and zinc (5.47±0.32 to 6.72±0.32mg/100g). Similarly, analysis of the vitamin profile revealed a maximum concentration of vitamin C (262.27±2.72mg/100g) and A

(516.95±5.31mg/100g) in the berry portion of Shighar cultivar whereas., minimum concentration (33.66±0.85 and 28.47±1.18 mg/100g) was in seed portion of SBT from Gilgat.
The fatty acid profile of different components revealed, an abundance of unsaturated fatty acids in seed (63.51±2.74 to 70.06±3.05%), berry (49.54±2.05 to 55.68±2.57%) and whole berry (52.02±1.73 to 58.11±1.84%). Among the unsaturated fatty acids (UFAs) the values of mono- and poly-unsaturated fatty acids were highest in the berry portion (47.23±13.2 to 52.41±1.78%). with the lowest concentration in seed portion (22.25±1.34 to 23.90±1.41%). Furthermore, oleic acid (7.94±0.27 to 9.68±0.66g/100g), palmitoleic acid, (10.08±0.52 to 12.39±0.66g/100g), γ -linolenic acid (16.11±0.42 to 19.15±0.50g/100g) and linoleic acid (22.87±1.07 to 28.11±0.80g/100g) were most abundant unsaturated fatty acids in the seed portion whereas vaccenic acid, (3.33±0.22 to 5.32±0.50g/100g), oleic acid (17.87±0.34 to 18.55±0.43 g/100g) and palmitoleic acid (26.60±0.65 to 28.54±0.86g/100g) were dominating in berry.
The different components of SBT were rich in essential and non-essential amino acids in sufficient quantity. The SBT seed portion exhibited threonine in a large proportion (6.63±0.54 to 10.40±0.65g/100g). Likewise, threonine (2.94±0.25 to10.33±0.73g/100g) and methionine (5.75±0.24 to 15.76±0.73g/100g) were abundant in the berry portion. Among the non-essential amino acids cysteine was high in seed (13.09±0.94 to 22.35±1.38g/100g) and berry (14.95±0.79 to 19.11±1.01g/100g) The total contribution of essential amino acids was 48.76 to 62.55, 53.40 to 63.24 and 53.69 to 63.12% in seed, berry and whole berry portions, respectively. Similarly, the non-essential amino acids were higher in the berry portion (58.81±4.39%) and lower in the seed portion (54.82±7.95%).
The assessment of bioactive components of SBT berry, seed and whole berry portions revealed the highest total phenolic content (22.41±2.22 to 73.83±3.36mgGAE/g) in berry portion whereas total flavonoid contents were higher in seed (15.69±1.74 to 30.52±2.63mgQE/g). The highest antioxidant activity (6.46±0.43 to 9.38±0.97mmolTrolox/100g) was in the berry portion. followed by whole berry (5.80±0.44 to 8.44±0.85mmolTrolox/100g) and seed (2.88±0.40 to 4.28±0.45mmolTrolox/100g).

Supplementary Data

Proximate composition

The proximate composition of different components of sea buckthorn berries collected from different locations showed the highest moisture content in B-Sh whereas the lowest in S-Gi (Table 1). The protein contents were observed higher in S-Sh whereas, lowest was in B-Gh. The highest values of crude fat were observed in B-Gi whereas S-Kh showed lowest content. The crude fiber in seed was ranged from 5.46±0.31 to 12.31±0.89%. Among different components, the ash content varied from 1.64±0.15 (S-Gh) to 3.61±0.35% (B-Gh). The highest value of NFE was observed in B-Gi and the lowest in S-Sh.
The variations in chemical composition of sea buckthorn seed were might be due to difference in genetic makeup, climatic conditions and agronomic practices. Previously; Pilat and his colleagues evaluated the chemical composition of individual morphological parts of the sea buckthorn fruit (*Hippophaë rhamnoides* L.) and showed that seed portion on dry weight basis contain moisture content (10.50±0.24%), crude protein (29.34±1.14%), crude oil (13.00±1.32), crude fiber (12.99± 0.32%) and ash (2.37 ±0.21%) [38]. In another study, Bal *et al.*, (2011) has reported 4.67% moisture content in the seed [39]. Moreover, Uransanaa *et al*., 2003) has reported 37.79% protein content in the seed [34]. Similarly, Khan *et al.,* (2005) has reported 16.56% moisture content, 37.79% crude protein and 7.95% crude fat in seed portion [30].
Regarding the composition of berry, a study reported Ciesarová *et al*., (2020), sea buckthorn berry contain 60-85% moisture content and 36.8 to 42% nitrogen free extract [26]. In another study, Jaroszewska *et al.*, (2018) found 8.6-10% crude fiber and 4-4.1% total ash in berry [23]. Previously, Piłat *et a*l., (2014) has reported 7.72% fiber in the dry matter [38]. The chemical composition of Mongolian sea buckthorn performed by Buya *et al.,* (2012) has revealed the presence of various levels of moisture (4.67±0.64%), crude protein (4.49±0.5%), crude fat (50.3±0.73%), crude fiber (9.17±0.50%), total ash (1.7±0.04%) and NFE (29.67±0.35%) [28]. Ranjith *et al*., (2006), has

investigated three species of SBT (*Hippophae rhamnoides, H. salicifolia and H. tibetanacollected*) collected from different regions of Indiaian Himalayas and witnessed 1.99±0.23 to 3.09±0.13% moisture content, 1.53±0.15 to 3.61 ± 0.25% crude fat and 4.49±0.5% crude protein content in berry portion [14].

The higher moisture content is an undesirable characteristic for a powered product as it may lead to sogginess and product deterioration due to microbial infestation. The possible reasons of great variation in moisture content were might be the geographical location, rate of annual rainfall, variations in drying process and storage conditions. The highest moisture content 13.16% in SBT from Shighar was might be due to high rate of annual rain fall (933mm) in this particular region. Overall, the significant variations in moisture content of all components of berry were observed according to their geographical locations.

Protein is an essential need of the people living in developing countries due to high rate of underweight, stunting and wasting. The components of sea buckthorn berry are rich in protein; containing all essential amino acids. Protein content is marginally higher in seed portion than that of berry. Variations in protein contents among berries of different locations were might be due to environmental aspects (salinity, alkalinity diseases attack and temperature), level of fertilization, geographical location, weather conditions and seasonality.

Fat is one of the most important component of SBT berry and seed due to its medicinal role. The components of SBT were observed to contain significant quantity of crude fat carrying all essential fatty acids, fat soluble vitamins (A, D and E) and lipophilic bioactive compounds. The difference in fat content in SBT of different geographical locations was due to environmental conditions. Berry is the major oil containing component of SBT. Ranjith *et al*., (2006) reported the variation in pulp content of different species (1.53±0.15 to 3.61±0.25% FW) [14]. It is obvious from the results that seed contain marginally higher fiber content than other components of fruit. Moreover the role of fiber intake is pivotal against CVD's. The difference in composition of berry regarding fiber was might be due to genetic diversity and environmental conditions.

The ash content is a valuable component of SBT while the berry contained higher ash content than seed. The variations in ash content might be due to difference geographical location, genetic makeup, and environmental conditions. Nitrogen-free extract (NFE) provide an estimation of water-soluble polysaccharides *e.g.*, sugars and starch in foods. Fortunately, SBT seed and berry portions were observed the rich source of NFE with marginally higher concentration in berry. NFE values may vary due to cultivars differences.

Table 1. Means for proximate composition (%) of seed portion of sea buckthorn

Geographical Locations	Proximate composition (%)					
	Moisture	Crude Protein	Crude Fat	Crude Fiber	Total Ash	NFE
S-Kh	9.63 ± 0.36^{b}	22.83 ± 0.94^{ab}	11.30 ± 0.34^{bc}	12.31 ± 0.89^{a}	2.20 ± 0.23^{ab}	42.98 ± 1.14^{ab}
S-Sh	11.89 ± 0.72^{a}	23.60 ± 0.78^{a}	13.63 ± 0.60^{a}	11.35 ± 0.70^{b}	2.60 ± 0.39^{a}	39.88 ± 0.98^{b}
S-Gi	8.25 ± 0.31^{b}	21.32 ± 0.81^{bc}	12.02 ± 0.50^{c}	10.88 ± 0.33^{b}	1.83 ± 0.29^{bc}	45.64 ± 1.54^{a}
S-Gh	9.79 ± 0.52^{b}	20.37 ± 0.63^{c}	12.94 ± 0.29^{ab}	09.27 ± 0.59^{c}	1.64 ± 0.15^{c}	42.54 ± 1.72^{a}
B-Kh	11.30 ± 0.38^{b}	11.92 ± 0.39^{ab}	22.86 ± 0.75^{a}	7.74 ± 0.67^{a}	2.72 ± 0.18^{b}	45.64 ± 1.60^{c}
B-Sh	13.42 ± 0.45^{a}	12.31 ± 0.42^{a}	20.53 ± 0.55^{b}	6.02 ± 0.45^{bc}	2.85 ± 0.29^{b}	46.19 ± 1.29^{b}
B-Gi	9.44 ± 0.28^{c}	10.09 ± 0.38^{bc}	22.30 ± 0.63^{a}	5.46 ± 0.31^{c}	2.99 ± 0.30^{b}	49.70 ± 0.55^{a}
B-Gh	10.66 ± 0.15^{b}	9.98 ± 0.240^{c}	18.33 ± 0.32^{c}	7.25 ± 0.55^{ab}	3.61 ± 0.35^{a}	46.37 ± 1.02^{b}
W-Kh	11.02 ± 0.37^{b}	13.75 ± 0.57^{a}	19.96 ± 0.76^{b}	8.34 ± 0.40^{a}	2.71 ± 0.16^{b}	45.19 ± 1.86^{b}
W-Sh	13.16 ± 0.44^{a}	14.21 ± 0.72^{a}	17.06 ± 0.63^{c}	6.91 ± 0.14^{bc}	3.50 ± 0.50^{a}	45.13 ± 1.79^{b}
W-Gi	09.24 ± 0.29^{c}	11.98 ± 0.65^{b}	20.56 ± 0.81^{a}	6.37 ± 0.55^{c}	2.80 ± 0.42^{b}	49.02 ± 0.91^{a}
W-Gh	10.51 ± 0.35^{b}	11.73 ± 0.46^{b}	21.94 ± 0.89^{a}	7.94 ± 0.50^{ab}	3.34 ± 0.13^{a}	45.36 ± 0.96^{b}

Means carrying same letter in a column differed non-significantly ($p>0.05$)
Means±S.D.
S-Kh=Seed portion of SBT berry from Khaplu; S-Sh=Seed portion of SBT berry from Shigar; S-Gi= Seed portion of SBT berry from Gilgat;S-Gh= Seed portion of SBT berry from Ghizer; B-Kh=Berry portion of SBT berry from Khaplu; B-Sh=Berry portion of SBT berry from Shigar; B-Gi= Berry portion of SBT berry from Gilgat;B-Gh=Berry portion of SBT berry from Ghizer; W-Kh= Whole berry of SBT from Khaplu; W-Sh=Whole berry of SBT from Shigar; W-Gi=Whole berry of SBT from Gilgat; W-Gh=Whole berry of SBT from Ghizer

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