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DETERMINATION OF OPTIMUM HARVESTING TIME FOR VITAMIN C, OIL AND MINERAL ELEMENTS IN BERRIES SEA BUCKTHORN (*HIPPOPHAE RHAMNOIDES*)

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Abstract

Sea buckthorn a magic plant from Northern areas of Pakistan has multiple uses against various ailments, soil enrichment and environmental purposes. The fruit berries are rich in vitamin C, vitamin A, vitamin E, essential oil, Phytosterol and minerals (Fe, Ca, P, Mn and K). The micronutrient like vitamin C, oil, phosphorus, magnesium and calcium contents in fruit berries of cultivated sea buckthorn (*Hippophae rhamnoides.* L. spp. *sinensis*) harvested at three ripening times were determined using biochemical analysis techniques. Harvesting at different stages of fruit ripening was the primary factor determining maximum expression of these biochemical constituents. Biochemical contents were determined at three fruit developmental stages i.e., unripened stage, medium stage and at full-ripened stage. During this study a decline in vitamin C contents was observed along with the fruit ripening. The oil contents in both seed and pulp increased with fruit ripening. Similarly, the mineral contents like magnesium, calcium and phosphorus contents increases with the fruit ripening in sea buckthorn.

The main idea was to identify the maximum expression of biochemical at different stages of fruit maturity. It is concluded that it is better to harvest fruit berries at medium stage of fruit ripening when maximum vitamin C is present. For maximum oil and mineral contents fruit must be harvested at ripening stage. The fruit mesocarp is the area where all genes related with micronutrients are active at one time i.e., when fruit is maturing, hence the characterization of gene expression activities at this stage may help in the isolation of these genes for future commercial use.

Introduction

Sea buckthorn is a general term given to the shrub or small tree of the genus *Hippophae*. The genus belongs to the family Elaeagnaceae that consists of six species and ten subspecies. The most economically important one is *Hippophae rhamnoides* L., commonly known as sea buckthorn (Rongsen, 1992). It is resistant to cold, drought, salt and alkali. Studies have shown that the fruit of Sea buckthorn is a storehouse of Vitamins and important bioactive substances (Xurong *et al*., 2001). The vitamin C contents are 5 to 100 times higher than any other fruit or vegetable known. The pulp also contains high quality oil which is regarded to be very important for its medicinal value (Rongsen, 1992; Lebeda, 2004).

Sea buckthorn fruit is rich in nutrients such as carbohydrates, organic acids, amino acids and vitamins. The vitamin C concentration in berries varies from 360mg/100g of berries for the European subspecies *rhamnoides* (Rousi & Aulin, 1977; Plekhanova, 1988; Wanlberg & Jeppsson, 1990; Yao *et al*., 1992) to 2500mg/100g of berries for the

Chinese subspecies *sinensis* (Yang *et al*., 1988). Vitamin C in fruits generally ranged from 200 to 1500mg/100g which is 5 to 100 times higher than any other fruit or vegetable known. The only sub species found in Pakistan is *Turkestanica* (Shah *et al*., 2007; Rongsen, 1996). A few scientists reported about the biochemical constituents in sea buckthorn ecotypes of Pakistan (Shah *et al*., 2007, Sabir *et al*., 2005) but the studies were mainly based on the samples collected at the stage when fruits were matured. The biochemical constituents vary in their quantity with different times of fruit harvesting. Harvesting dates of berries is one of the factors affecting vitamin C contents in sea buckthorn (Kallio *et al*., 2002). Sea buckthorn is a potent multipurpose plant distributed throughout Northern Areas of Pakistan as wild populations. Despite of the importance of plant it has been underutilized in Pakistan. A project has been started in the department of Plant Breeding and Molecular Genetics, Faculty of Agriculture Rawalakot, University of Azad Jammu and Kashmir for multipurpose utility of sea buckthorn by using its biochemical strengths and plant based genes source for biofortification of maize and wheat. The nutritional and biochemical importance of shrub is a well documented fact now. The study will ensure the identification of optimum harvesting time of sea buckthorn berries for different biochemical constituents including vitamin C, Oil contents, Fe, Ca, Mg, and P to be better exploited for commercial purposes and also to extract functional mRNA of important micronutrients for cDNA synthesis for future program of biofortificatoin of cereals.

Materials and Methods

The plant material used in the study was obtained from the cultivated berries of *Hippophae rhamnoides* from the nursery at Faculty of Agriculture Rawalakot. The *rhamnoides* berries were picked from four separate plants at three different stages starting from end of July 2008 to the beginning of September 2008. The samples were taken on the following dates, before they were subjected to -20^{o}C.

1st sampling 25th July 2008
2nd sampling 15 August 2008
3rd sampling 5th September

Three stages were given names stage 1, 2 and 3 respectively for interpretation of results. Determination of ascorbic acid, oil contents, Ca, Mg, P from the fruit samples of sea buckthorn was carried out under standard conditions using method as described by AOAO, (1984), Shah *et al*., (2007) and Sabir *et al*., (2005). Three readings were taken for the ecotype and means were computed. SD were computed using Microsoft excel software.

Results and Discussion

The present studies compared fruit berries of Sea buckthorn (*Hippophae rhamnoides* L., ssp. *Sinensis*) in term of the contents (vitamin C, oil and minerals) and changes of these compounds during harvesting period (Figs. 1-6). When the berries of Sea buckthorn were compared on the basis of vitamin C at different stages of fruit ripening, a wide range of variation was observed between these three stages. A decline in vitamin C contents was observed along with ripening stages. Highest concentration of vitamin C was observed during the 2nd stage (399.0 mg %). The concentration of vitamin C decreased during the ripening stage and found to be 263.05 mg% for 3rd stage. The vitamin C contents obtained during 1st stage were slightly lower than vitamin C contents at 3rd stage of ripening and were 294.66%. Figure 1 presents the vitamin C contents in berries harvested at different time periods. It is concluded that maximum expression of vitamin C occurs at medium stage and vitamin C contents decrease with ripening.

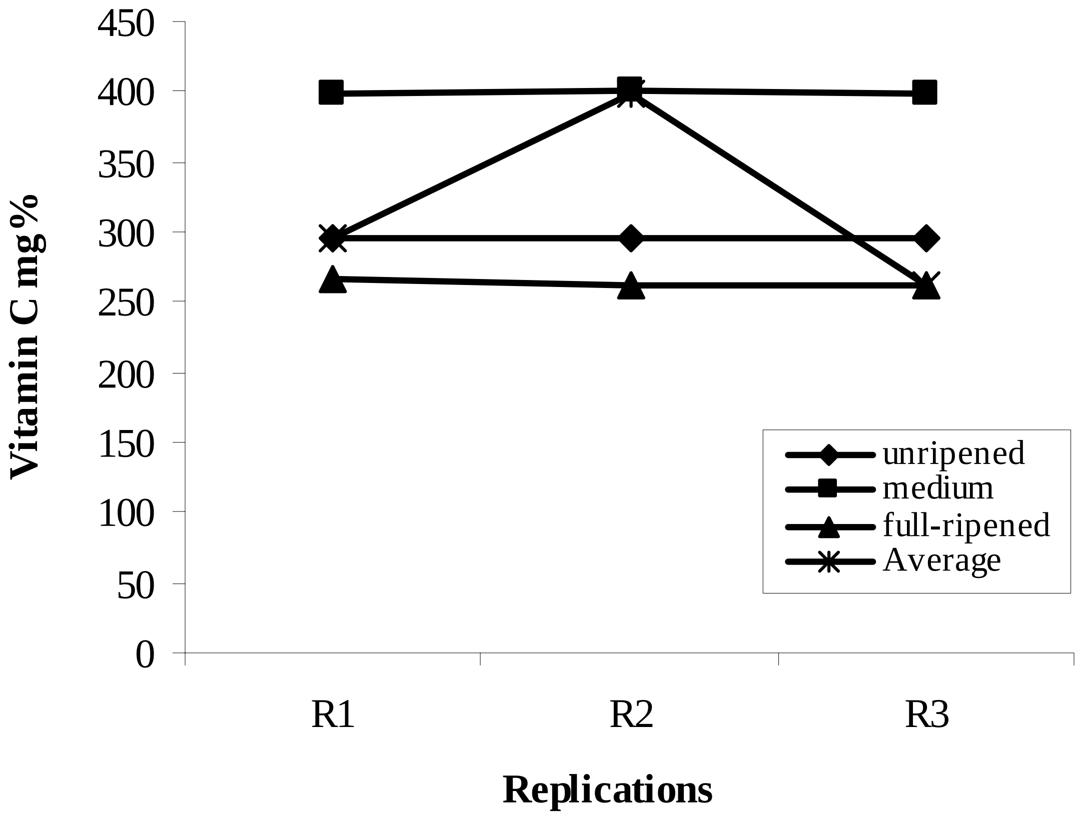

Fig. 1. Vitamin C contents at unripened, medium and fully ripened stage.

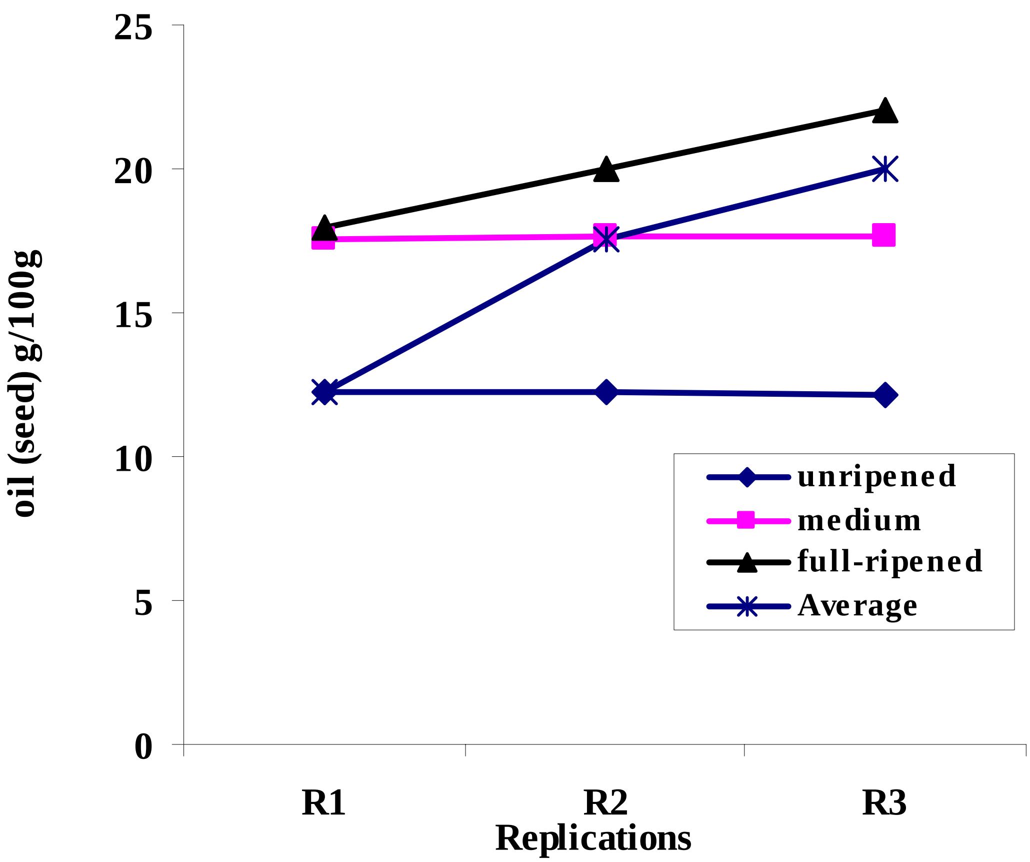

Fig. 2. Oil content %age seed at unripened, medium and fully ripened stage.

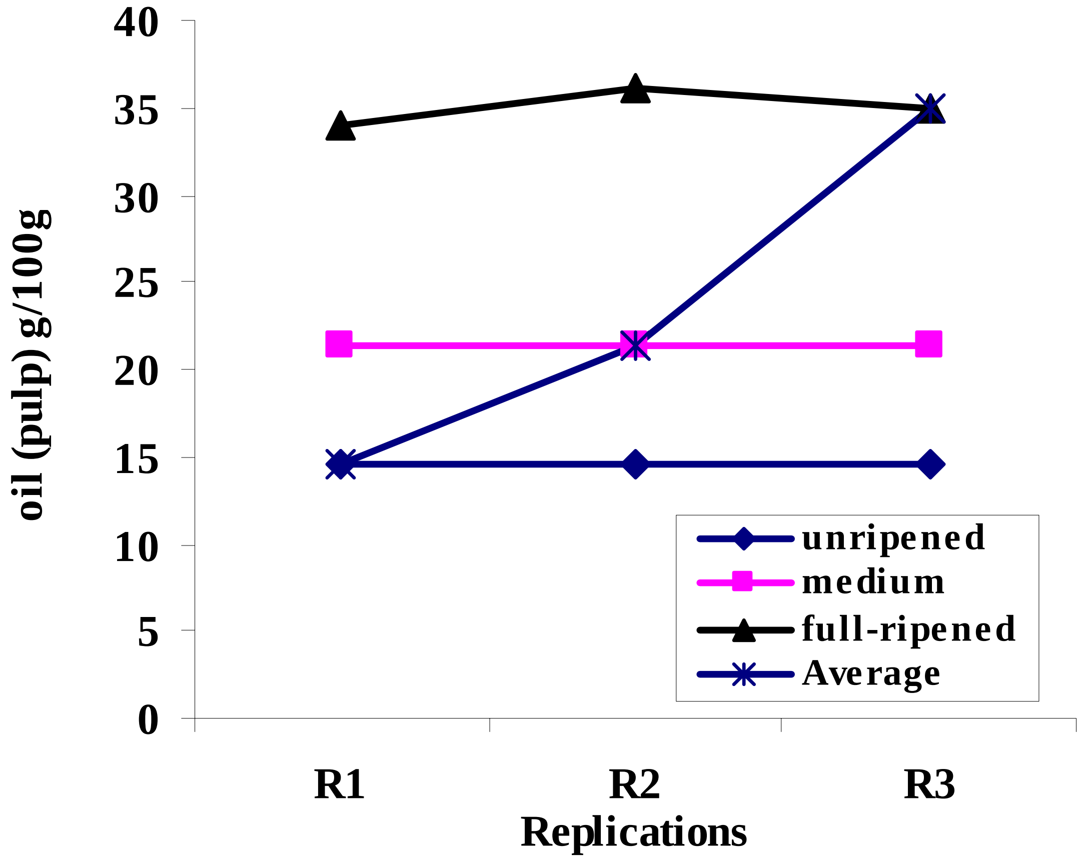

Fig. 3. comparison of oil contents %age pulp at unripened, medium and fully ripened stage.

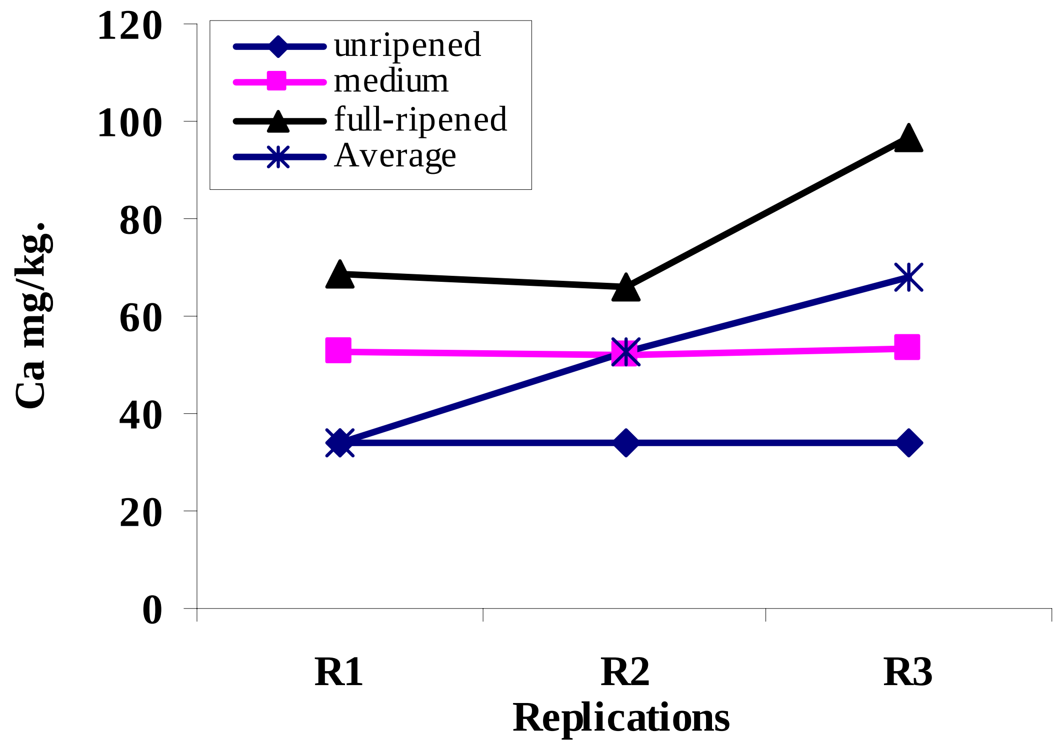

Fig. 4. Calcium contents at unripened, medium and fully ripened stage.

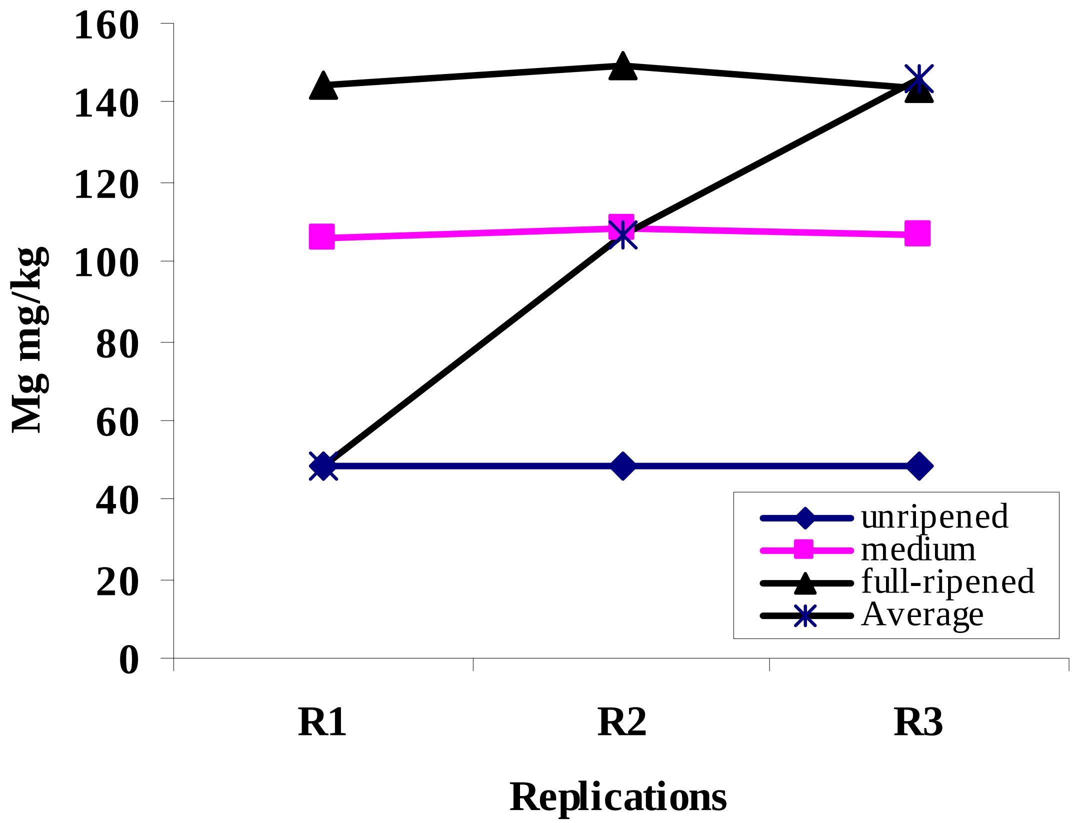

Fig. 5. Magnesium contents at unripened, medium and fully ripened stage.

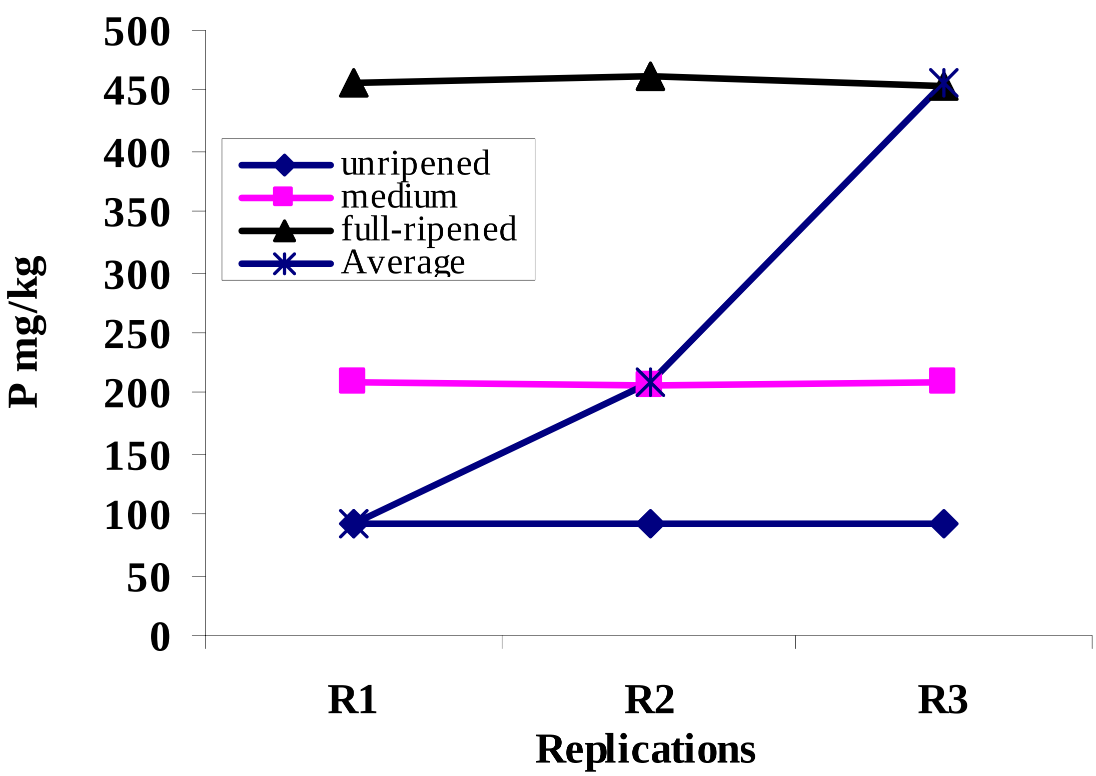

Fig. 6. Phosphorus contents at unripened, medium and fully ripened stage.

Antonelli *et al*., (2003) also reported that vitamin C contents decreased with fruit ripening. According to them the rate of decline however, was much higher at an early stage of ripening than in August and continued even after full ripening was reached. Similar, gradual decline in vitamin C along the entire period was observed. The rate of decline however was much higher at ripening stages and lower at medium stage and unripened stage. Kallio *et al*., (2002) reported that vitamin C contents in berry juice followed a decreasing trend during the harvesting period from late August to early December. Rousi and Aulin (1977) reported a decreasing trend in vitamin C contents, accompanied by a steady increase in the fresh weight of the berries. Tang & Tigerstedt (2001) reported that vitamin C contents of sea buckthorn berries also varied between different growing seasons.

We found that the Vitamin C contents were higher at the medium stage when the berries were harvested during the mid August, which coincided with the findings of Zhang *et al.,* (2006) who reported that the acid contents increased at the beginning and middle of fruit growth and decreased after the development of fruit color. Vitamin C content was highest at the ripening stage of the fruits and decreased thereafter.

The oil contents of sea buckthorn berries also vary significantly at different stages of ripening in both seed and pulp. The highest concentration of oil contents in seed was however detected during the 3rd stage (20.02%). A slightly lower concentration (17.6%) of oil in seed was detected during the 2^{nd} stage of fruit development. However, the lowest concentration (12.20%) of oil in seed was detected during the unripened stage. The results of oil contents are shown in Fig. 2 with average value. Ma *et al*., (1987) reported that the oil contents found in seeds of subsp. *sinensis* of Northern mountain areas of China was 10.2-12% which is nearly in equal amount of our oil contents percentage determined in our investigation at different stages of ripening. Oil contents in softer part (pulp) were also found to be significantly different at different stages of ripening. The highest concentration of oil (35.04%) in pulp was detected during the ripening stage. A slightly lower concentration of oil (21.36%) was found during medium stage. The lowest concentration (14.63%) was detected during the unripened stage. A clear comparison of oil contents in pulp at different stages is shown in Fig. 3. The oil contents percentage found in both pulp and seed during the ripening stages were higher than the other two stages of ripening. Our investigations are in agreement with the observation of Sabir *et al*., (2005) who reported seed oil 7.69-13.70% and oil in dried pulp 19.20-29.10%. Similarly Shah *et al*., (2007) showed the values of oil contents in the range of 18.20-43.50% and 7.03-12.86% in sea buckthorn berry pulp and seeds respectively.

Sea buckthorn berries are rich in multiple mineral elements. There are at least 24 chemical elements present in sea buckthorn juice e.g., nitrogen, phosphorus, iron, manganese, boron, calcium, aluminum, silicon and others. The mineral elements investigated during these studies were phosphorus, magnesium and calcium. Sea buckthorn berries are also rich in Ca contents. The Ca contents of ecotypes of Sea buckthorn were significantly different at different stages of ripening as shown in Fig. 4. The maximum Ca contents (68.28mg/kg) were found during the ripened stage, which is significantly different and higher than the other two stages of ripening. During unripened stage Ca contents was found to be 33.71mg/kg. The Ca contents observed during the medium stage were also significantly different (52.82mg/kg). Sabir *et al*., (2005) found calcium contents in the range 0.7-1.25 g/kg from the berries harvested from Northern Areas, which is slightly lower than over investigation. It may be due to the fact that our samples were taken from plant populations established at Rawalakot and environmental conditions at Rawalakot may increase the calcium contents in sea buckthorn berries.

When ecotype was compared on the basis of Mg contents, a significant variation was observed during the different harvesting times as shown in Fig. 5. The lowest concentration of Mg was found during the unripened stage (48.06mg/kg). The Mg contents found during the medium stage were significantly different and higher (106.92mg/kg) than the Mg contents expressed during unripened stage. The highest concentration (145.67mg/kg) of Mg was found during the ripened stage. Our studies on the magnesium contents agree with the findings of Sabir *et al*., (2005) who reported magnesium contents ranged for 139-240 mg/kg.

Sea buckthorn berries are also rich in phosphorus contents when ecotype was compared on the basis of mineral phosphorus during different ripening stages a significant variation was observed during these stages. The lowest concentration of phosphorus was found during unripened stage. Phosphorus contents found during the medium stage was significantly higher than the unripened stage. Phosphorus contents found during the ripened stage were higher than the phosphorus found during the other two stages of ripening. Phosphorus contents during this stage were 457.7mg/kg. The phosphorus contents found during the unripened and medium stage were 93mg/kg and 208.1mg/kg respectively. This variation in phosphorus contents at different stages was compared in Figure 6. Shah *et al*., (2007) reported similar findings.

Conclusions

The results of the present study suggested that mid-August was the best harvesting date for berries because maximum vitamin C contents were found during the medium stage of fruit ripening while mineral and oil contents were higher during fully ripened stage when fruits were harvested during September.

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