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VARIATION IN PHYTOCHEMICALS WITH RIPENING STAGES OF RUSSIAN OLIVE (*ELAEAGNUS ANGUSTIFOLIA*) VARIETIES GROWN IN CENTRAL HUNZA, GILGIT BALTISTAN

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ABSTRACT

Three varieties of Russian olive berries grown in central Hunza Valley were evaluated for phytochemicals analysis at different ripening stages (un-ripened, semi-ripened and fully ripened). Flavonoid, carotenoid and total phenolic contents were determined at each ripening stage. A uniform trend did not appear with ripening stages rather variable results were found. In Xharey variety, total phenolic contents were increasing whereas totalphenolic contents were slightly decreased However, carotenoids were remain constant. Likewise, total phenolic contents were decreased with the ripening conditions in Bulbulkiyanz variety. Whereas total flavonoid were highly increased with ripening which was estimated the highest among three varieties showed 0.409mg/g. Carotenoid contents also showed slight decreasing trend with ripening in Bulbulkiyanz variety. In Hook variety, total phenolic contents were increased ripening stage at highest concentration of 0.438mg/g. Total flavonoids showed decreasing trend with ripening while carotenoid contents were highest amongst the three varieties at un-ripened stage.

KEY WORDS: Russian olive varieties and phytochemicals.

INTRODUCTION

Russian olive (*Elaeagnus angustifolia)* belongs to family *Elaeagnaceae* cultivated in Himalayan ranges and extended to Europe because of its ability to grow in diverse environmental conditions. Klich (2000) found that Russian olive contains 3.4% of amino acids, 6.76 % of protein, 5.43 % of fat and 8.69 % of fiber. This also suggests that it is rich in nutrition and has healthy food properties. Ahmedani *et al*. (2007) reported that in last two decades researchers have admitted the importance of fruits not only as source of food but also for their probable medicinal value. Folk uses fruits evident as an effective medicine and shown potential to resists against diseases. Besides, they further reported that Russian olive fruits are important source of compounds creating interest for pharmacy and food industry. According to Mirhydar (2003) it provides sugars like sucrose and fructose. Sherwali and Surrya (2007) fruits of Russian olive are edible and medicinally used for curing urinary diseases, gastric disorders and diarrhea, nausea, vomiting, jaundice, asthma, flatulence, dysentery and liver problems. According to Mirhydar (2003) elaborated the beneficial effects of fruits, in particular soft fruits are associated to have antioxidants like Vitamin C, carotenoids and other Phenolic compounds for instance flavonoids and phenolic acids. It has wound healing effect and shows some antioxidant activity and its use in Gilgit-Baltistan as Joshanda ingredient for curing cough and cold. Likewise, some other researchers like Akesnov and Akesnov (2000) reported that therapeutic and prophylactic properties of Russian olive and have been noticed in folk medicine in many Asian and trans-Caucasus countries and have been used as cure for gastro-intestinal tract disease in these countries because of their astringent anti-inflammatory and encapsulating activity. Russian olive fruits are also used as cholegogics (for bronchitis), diuretics (for edema), anti-helmintics and for vitamins. Fruit tincture exhibits hypotensive and slight analgesic action. Central Asian people make baby food in powder form from pericarp of Russian olive fruit

MATERIAL AND METHODS

Sampling

Three local varieties of wild grown Russian olive, (Vernacular names "Xharey", "Bulbulkiyanz" and "Hook") were taken randomly from central Hunza. Samples were taken at three ripening stages (un-ripened, semi-ripened and fully ripened) with 25 days time interval from October to November, 2013. Fruits were collected from unevenly distributed population. Fruits were harvested into polyethylene bags and were taken to Food Technology Laboratory at the Dpartment of Agriculture and Food Technology, Karakorum international University Gilgit in 2013. The samples were oven dried till constant weight and ground into powder for subsequent phytochemical evaluation.

Preparation of extract

Powdered Russian olive berries fruit were suspended in methanol for 48 hours at room temperature and was filtered. The concentrates were collected and dried in rotary evaporator and kept in dark at 5 °C until analysis. The extract was diluted with methanol (0.5 mg/ml).

Preparation of standard curves

Standard curves were prepared by following procedures for total phenolics, total flavonoids and carotenoids with gallic acid(2mg/ml, 4mg/ml, 6mg/ml, 8mg/ml and 10mg/ml), rutin (0.1mg/ml, 0.2mg/ml, 0.3mg/ml, 0.4mg/ml, and 0.5mg/ml) and β-carotene (0.05, 0.10, 0.15, 0.2 and 0.25 mg/ml) as standard respectively with little difference. Various concentrations were made in ascending order. Varian UV spectrophotometer was used for Spectrophotometric analysis of standard as well as samples.

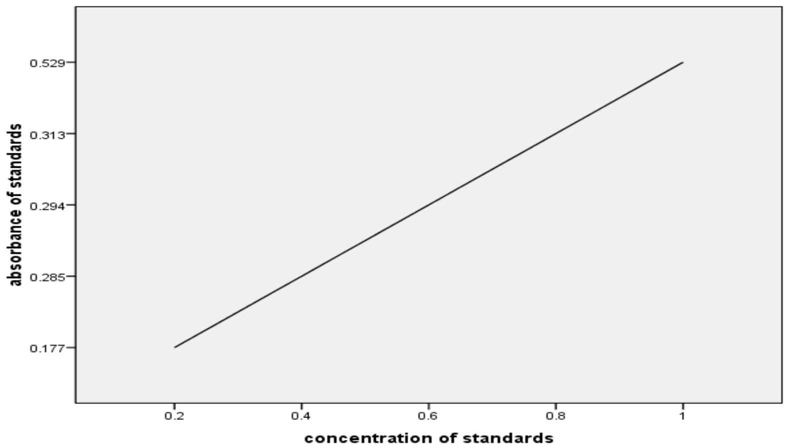

Figure 1-Standard curve for Gallic acid

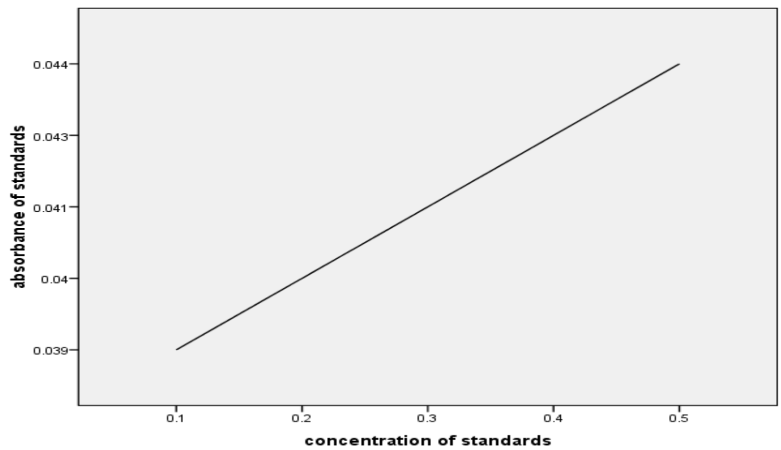

Figure 2-Standard curve for total Rutin

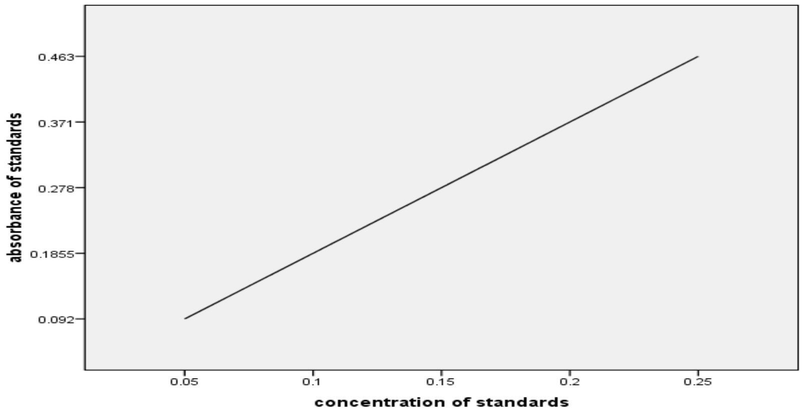

Figure 3-Standard curve for carotene

Determination of total phenolic content

Total soluble phenolics were determined using the Folin-Ciocalteu reagent method with little modifications using gallic acid as an internal standard. The fraction sample (1 ml, 0.5 mg/ml) was diluted with distilled water (4.6 ml) in a volumetric flask. Folin-Ciocalteu reagent (1 ml) was added and mixed thoroughly. After 3 minutes 3 ml of 2% sodium carbonate solution was added and the mixture was allowed to stand for 2 hour with intermittent shaking. The absorbance was measured at 760 nm and the concentration of total phenolics in the extracts was estimated as milligrams of gallic acid equivalent per gram of dry matter. All tests were done in triplicate.

Determination of total flavonoids content

Total flavonoids were measured by the following method. 1 ml of fruit extract (0.5mg/ml) was successively mixed with 4ml of water, 0.3ml of 10% AlCl3 and 2ml of 1.0 M NaOH. Finally, 2.4ml of water was added to reaction flask and mixed well and absorbance of the reaction mixture was measured at 510nm. Rutin was used as internal standard. All measurements were done in triplicate.

Total carotenoid determination

Total carotenoids were measured as per standard procedure. 1 ml sample was homogenized with 20ml of methanol: petroleum ether (1:9, v/v) and mixture were transferred to separating funnel, the top layer was transferred to volumetric flask and then total volume was made up to 20 ml with petroleum ether. Total carotenoid content was measured by a spectrophotometer at 450 nm and results were expressed as beta-carotene equivalents (mg/g of dry weight).

Data Analysis

Data obtained was statistically analyzed with the help of SPSS 16 (IBM) and the Two ways ANOVA was applied for factorial design at $p< 0.05$ taken as significant.

RESULTS AND DISCUSSION

Variation and quantification of phytochemical components at different ripening stages of Russian olive varieties has been studied. Many studies have been carried out on the variation in fruit quality at different ripening stages of various fruit species. The efficacy of phytochemicals and nutrients may vary in dietary fruits and vegetables with respect to variety, harvesting time, origin and climatic conditions.

Total phenolic content

The results show that all three varieties do not have same trend with total phenolics throughout the ripening process. The statistical analysis for total phenolic content showed highly significant results ($p< 0.01$). However, every variety exhibits different behavior in having total phenolic concentrations with the progression of ripening. Maximum concentrations of total phenolic content were observed in Hook variety at all the ripening stages as 0.282 ±0.016mg/g, 0.366 ±0.011mg/g and 0.438 ±0.001mg/g GAE respectively (Figure-1). Hook variety showed increasing trend in Total phenolic contents as ripening proceeds. Xharey variety showed similar increasing trend with total phenolic content so as ripening proceeds. It was seen that total phenolics accumulate with procession of ripening in Hook and Xharey variety. The ripened stage showed the concentration up to 0.292 ±0.0046 mg/g at mature stage (Figure-1). Whereas, the other two varieties showed decreasing trend in total phenolic content so as ripening progress. The variation in total phenolic content in flesh tissues of fruits is because of chemical reactions, enzymatic alterations in phenolics, hydrolysis of glycosides by glycosidase, oxidation of phenols by phenol oxidase polymerization of free phenols (Remorini *et al.*, 2008). The least amount of total phenolic content was observed to be 0.185mg/g at ripened stage (Figure-1). The best harvesting stage with respect to total phenolic content was ripened stage in Hook variety. Total phenolic content observed in this study are less as compared to observations in sea buckthorn belonging to Elaeagnaceae family, which range from 54.4 to 86.4mg/0.25g GAE (Gupta and Kaul, 2012). The obtained results were also in fewer concentrations than found by Miletic *et al.*, (2012). Raspberry is also containing higher quantity of total phenolic contents 864.4 to 1246.9µg/g GAE.

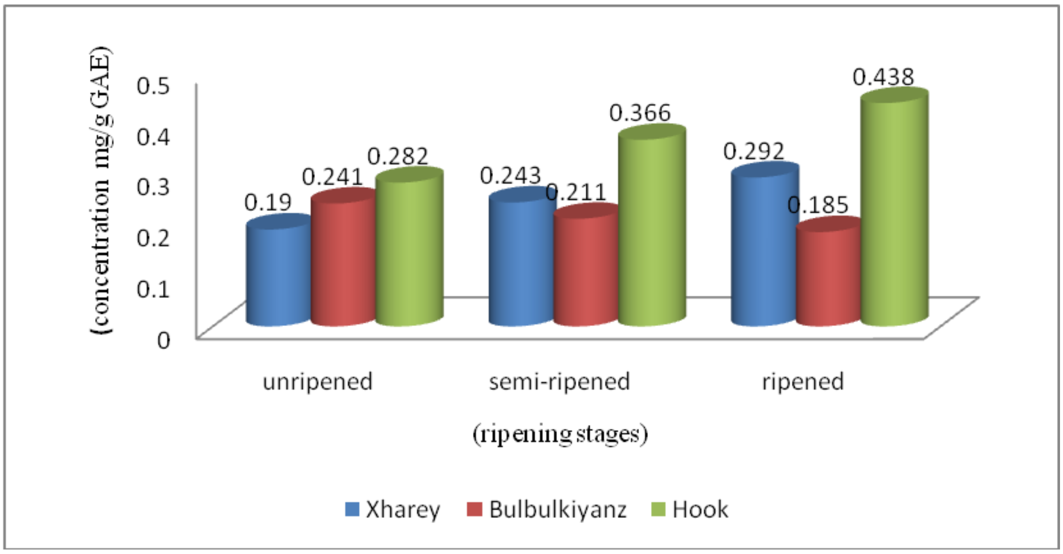

Figure 1- Total phenolic content of Russian olive berries at three different ripening stages.

Total Flavonoids

The flavonoid contents in Bulbulkiyanz variety of Russian olives were highest at full ripened stage with 0.409mg/g concentration (Fig-2). This variety showed an increasing trend with ripening. Xharey variety showed a little decrease in overall flavonoid content as compared to Bulbulkiyanz variety. There was decreasing trend in flavonoid concentration in Hook variety unlike Xharey and Bulbulkiyanz. The lowest concentration 0.357mg/g was observed in Hook at ripened stage. Xharey variety did not show any decreasing or increasing trend rather it exhibited almost uniform trend in concentration of total flavonoids with ripening. This study suggests, the best variety for flavonoid is Bulbulkiyanz at ripened stage. Flavonoid contents were less than reported by Lashmanova *et al.* (2012) for *Elaeagnus conferta* but more than *Helicteris isora* i.e. 0.33mg/g. The results also show that flavonoid contents were less than that of raspberry, bilberry and blackberry varieties which range from 1.433 to 7.163 mg/g RE as found by Stajcic *et al.* (2012). (Liu *et al.*, 2008 and Tabata *et al.*, 2008) reported that flavonoid concentration varies from plant to plant or even at different ripening stages and organs of the same plant. Higher flavonoid content in un-ripened fruit might be because in later stages of ripening different phenolic acids might have condensed to form complex phenolic compounds like tannins and lignin etc.

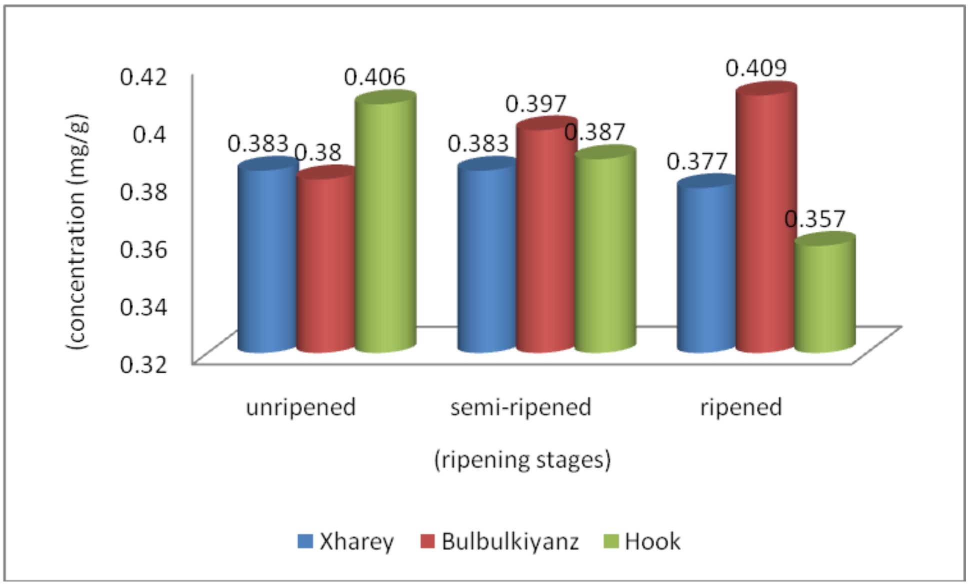

Figure 2-Total flavonoid content of Russian olive berries at three different ripening stages.

Carotenoid contents

The carotenoid content does not show any distinct trend in all varieties rather individual variety show its own trend (Fig-3). Hook and Xharey variety shows decreasing trend with ripening while Bulbulkiyanz variety shows uniform concentration of carotenoids at all three ripening stages with slight decrease in semi-ripened stage. This variety shows the least carotenoid concentration, 0.077mg/g. On contrary to this Bulbulkiyanz variety show decreasing trend as ripening proceeds. The highest concentration in this variety was observed to be 0.089mg/g. Hook variety has the highest concentration 0.098mg/g of carotenoids at un-ripened stage which decreases in semiripened stage to 0.062mg/g and shows increase in ripened stage to 0.083mg/g. Resulsts shows that the carotenoid content overall decreases with ripening in Hook variety. Total carotenoids observed in this study are lesser in concentration in comparison to cloudberry (2840 µg/100g), blue berry (2140 µg/100g), cranberry (200 µg/100g) and cowberry (140 µg/100g) reported by Lashmanova *et al.* (2012). Russian olive proves itself to be better source of total carotenoids in comparison to seabuckthorn which contains 0.0011 mg/g to 0.0142mg/g of total carotenoids. Carotenoids are well known for their beneficial role in human health.

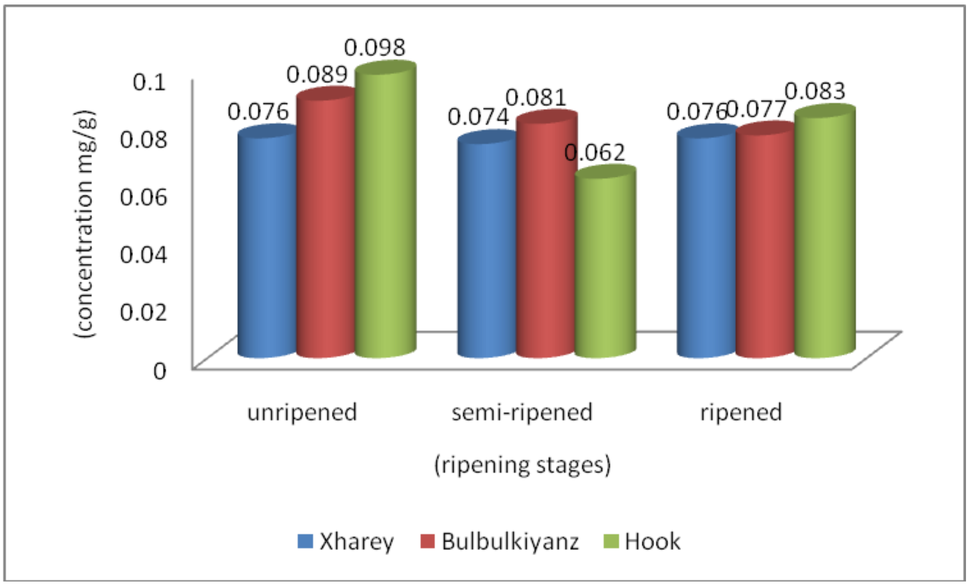

Figure 3-Total flavonoid content of Russian olive berries at three different ripening stages.

REFERENCES

1. Ahmedani, A., Hossseiny, J., Semnanian, S., Javan, M., Saeedi, F., Kamalinejad, M and Saremi, S (2007). Antinoceceptive and anti-inflammatory effects of Elaeagnus angustifolia fruit extract. J Ethnopharmacol, 72,287-92.
2. Akesnov, E.S and Aksenova, N.A (2000). Decorative plants in Russia, vol.1. Trees and Bushes. Encyclopedia of nature in Russia, ABF, Moscow, 305-308.
3. Klich M.G (2000). Leaf variations in Elaeagnus angustifolia relate to environmental
4. heterogeneity. Envexp botany, 44,171-83.
5. Lashmanova, Katya .A, Kuzivanova and Olga .A. Dymova Olga .V (2012). Northern berries as source of carotenoids. Biochem Polinica Vol. 59, No 1 ,133-134.
6. Liu, H., N. Qiu, H. Ding, and R. Yao. 2008. Polyphenols contents and antioxidant capacity of 68 Chinese herbals suitable for medicinal or food uses. Food Research International. 41:363-370.
7. Maga, J. (1978). Simple phenol and phenolic compounds in food flavor. CRC Crit. Rev Food Sci. Nutr.10, 323-372.
8. Mirhydar Indications of plants in the prevention and treatment of diseases, encyclopedia of plants, Tehran Islamic Farhang., H (1998).
9. SherWali, K and Surayya, K (2007).Ethnobotanical studies on useful trees and shrubs of Haramosh and Bagrote valleys in Gilgit Northern Areas of Pakistan. Pak j. bot. 39(3), 699-710.

10. Tabata, H., T. Katsube, T. Tsuma, Y. Ohta, N. Imawaka, and T. Utsumi. 2008. Isolation and evaluation of the radical-scavenging activity of the antioxidants in the leaves of an edible plant, Mallotus japonicus. Food Chemistry, 109: 64-71.
11. Remorini .A, Tavarani .S, Innocenti. E .Degl , Loreti .F, Massai .R, and Guidi .L (2008). Effect of rootstock and harvesting time on the nutritional quality of peel and flesh of peach fruits. Food Chemistry .110, 361-367.
12. Gupta .S.K., Goshal .M., Choudary .D and Mandal .p (2014). Assessment of antioxidant activity and polyphenolic content of couroptia guianensis during flower and flower and fruit maturation. International Journal of Recent Scientific Research, Vol. 5, Issue, 5, pp.940-947.