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Chemical Variability of Fatty Acid Composition of Seabuckthorn Berries Oil from Different Locations by GC-FID

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Abstract. For determining the chemical composition of seabuckthorn oil of different origins, samples of seabuckthorn berries (red and yellow varieties) were collected from different locations of northern areas of Pakistan. Among eight different fatty acids, palmitoleic acid (32.4%) and palmitic acid (36.52 %) were found to be the major fatty acids present along with other important fatty acids i.e., oleic acid (37.07%), linoleic acid (12.36%) and linolenic acid (0.73%). Quantities of unsaturated fatty acids were higher than that of saturated analogues.

Keywords: seabuckthorn berry oil, fatty acid composition, pharmacology

Introduction

Seabuckthorn (*Hippophae rhamnoides*) is a thorny shrub, found widely near streams, sandy places, mountainous and coastal areas of many Asian and European countries (Risto and Baoru, 2003). Natural habitat of seabuckthorn extends widely in China, Mongolia, Russia, India, Nepal, Pakistan, Ukraine, Great Britain, France and most parts of the northern Europe (Li and Shroeder, 1999). In Pakistan it is naturally growing in the northern areas of Gilgit and Chitral.

Scientists have carried out extensive research on seabuckthorn which has resulted in an improved understanding of the health effects and chemical composition of the berry (Yang and Kallio, 2002; 2001). The fruits of seabuckthorn are rich in carotenoids, lipids, ascorbic acid, tocopherols, sterols, flavonoids and triterpenes (Zhemin, 1990; Loskutova *et al*., 1989) and are a rich source of both aqueous and lipophilic antioxidants due to the presence of vitamins D and E, as well as enzymes such as various superoxide dismutase isoenzymes (Eccleston *et al*., 2002).

Seabuckthorn is rich in oil both in seeds (seed oil) and in the fruit soft parts i.e., flesh and peel (pulp oil). The observations reveal that the seeds contain around 10% of oil, whereas the oil content in the soft parts like pulp varies over a much wider range from 0.5 to 21%, largely depending on the varieties and the origin. The biological properties of fruits and oils of *H. rhamnoides* have been widely investigated. Oil extracted from fruit has regenerating antiinflammatory and photoprotective activity with promising applications in dermatology and cosmetics (Pintea *et al*., 2001). Seabuckthorn oil is known to regulate immune functions and antagonize the effects of immune suppressants (Yang and Kallio, 2001). In clinical cancer treatment, seabuckthorn has been used to reduce the immune suppressive and hematoxic effect of chemotherapy and radiation therapy.

Seabuckthorn seed and pulp oils combined have high level of beneficial fatty acids. The fatty acid profile of the oil showed that it contained nearly 90% unsaturated fat. It is high in both linolenic and linoleic acids. The high level of unsaturated fats makes seabuckthorn oil appropriate for decreasing the risk of heart diseases (Yang, 1995). Berry oil of seabuckthorn contains 35-40% of a 16 carbon monosaturated fatty acid called palmitoleic acid also known as omega-7. Palmitoleic acid is believed to possess potent antiviral, antibacterial and healing effects in humans (Parimelazhagan *et al*., 2005).

In medicinal industry about ten varieties of seabuckthorn drugs have been developed and are available in the form of liquid, powder, plasters, pills, liniments and aerosols etc. These drugs are used for treating burns, gastric ulcer, scales, oral mucositis, rectal mucositis, cervical erosion, radiation damage, skin ulcers caused by malnutrition and other damages relating to the skin (Ge, 1992). The most important pharmacological function of seabuckthorn oil is in diminishing inflammation, disinfecting bacteria, relieving pain and promoting regeneration of tissues. The chemical and phytochemical composition of seabuckthorn varies with the origin, climate and method of extraction. (Zeb, 2004; Beveridge *et al*., 1999). Present studies were carried out to analyze the physicochemical composition and fatty acid profile of oil of seabuckthorn berries collected from different altitudes of the northern areas of Pakistan by gas chromatography techniques because data in this respect is not available in the literature.

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Materials and Methods

Extraction of oil. Red and yellow varieties of berries of *H. rhamnoides* were collected from six different locations i.e., Nomal, Bagrote, Ghizar, Skardu Khas, Shigar and Khaplu and stored at -20 °C. Seeds were separated from frozen berries by pressing the juice and the residue was dried at room temperature. The pulp portion was also dried and oil was extracted by the cold press method yielding pure, chemical and solvent free oil.

The extracted oil was analyzed for refractive index, specific gravity, saponification value, unsaponifiable matter, acid value, iodine value and peroxide value according to standard methods (AOAC, 2000). Methyl esters of fatty acids were also prepared by standard methods (AOCS, 2000). Fatty acids were saponified by treating with 0.5M methanolic NaOH solution. After acidification with 2M HCl to pH 4-5, fatty acids were extracted with chloroform and the solvent was then evaporated under reduced pressure and temperature. The extracts were applied to TLC plates. Areas of silica gel corresponding to fatty acids were extracted from the plates with chloroform and then *trans*-esterified with boron trifluoride-methanol for 30 min at 80 °C. Methyl esters of fatty acids were dissolved in *n*-hexane and analysed by GC on a Perkin Elmer gas chromatograph (8410 series) using temperature programme with FID. Fused silica capillary column (25 m x 0.32 mm id, 0.15 µm film thickness) was used. Oven temperature was held at 65 °C for 5 min, then programmed at 5 °C/min up to 220 °C. The carrier gas was nitrogen with 1.5 ml/min flow rate. Injection and detector temperature was 250 °C. Split ratio was 1:20 and injection volume was 0.5 µl. Identification of the fatty acid methyl esters was carried out from the retention times compared to standards.

Results and Discussion

Physicochemical characteristics of oil of yellow and red varieties of seabuckthorn are given in Table 1. The values are mean of three independent readings.

Refractive index and optical rotation are very stable parameters and can be used for checking the identity of oils. Refractive indices of all the samples analyzed were almost the same, with insignificant differences. Maximum specific gravity of yellow variety was observed in samples collected from Nomal (0.951) while lowest value was observed in the samples from Skardu khas (0.855). The highest specific gravity of red variety (0.913) was observed in samples from Khaplu area while the lowest value (0.846) was observed in the samples collected from Ghizar. Acid values and peroxide values of the samples were quantified which were in accordance with the reported parameters and were within the range of reported data (Morsel and Steen, 2003).

Table 1. Physicochemical characteristics of pulp oil of seabuckthorn taken from six different locations

Variety	Nomal	Bagrote	Ghizar	Skardu Khas	Shigar	Khaplu
Refractive index						
Yellow	1.465	1.457	1.543	1.472	1.472	1.526
Red	1.469	1.458	1.543	1.473	1.523	1.531
Specific gravity						
Yellow	0.951	0.868	0.921	0.855	0.859	0.923
Red	0.871	0.860	0.846	0.851	0.905	0.913
Peroxide value						
Yellow	0.88	0.26	0.91	0.23	0.54	0.16
Red	0.81	0.60	0.63	0.23	0.50	0.37
Unsaponifiable matter						
Yellow	1.82	2.26	1.96	2.82	2.44	0.6
Red	3.30	0.80	2.20	2.40	3.60	1.6
Saponification value						
Yellow	192.20	229.20	210.0	186.20	186.90	189.0
Red	188.4	205.0	198.60	202.60	191.60	149.5
Iodine value						
Yellow	70	65	69	60	60	67
Red	71	68	70	61	64	65
Acid value						
Yellow	9.3	8.6	9.6	13.4	10.5	11.2
Red	9.25	9.5	8.00	11.0	12.35	11.35

Saponification value is the index of mean molecular weight of triglycerides comprising of fats. The highest saponification value was observed in oil from Bagrote (229.20), while the lowest was observed in the oil from Skardu Khas sample (186.20). Unsaponifiable matter mainly consists of soluble vitamins, pigments (i.e., carotene and lycopene), steroids, alcohols and hydrocarbons. Oil from Shigar had maximum value of unsaponifiable matter (3.60), while that from Khaplu had minimum value (0.6). It means that the former contained the highest amount of bioactive substances as compared to other samples. Iodine value is the measure of degree of unsaturation in oil or fat. Samples from Nomal had maximum Iodine value (70,71).

Fatty acid composition of seeds and berries of *H. rhamnoides* growing in different regions of the world has been extensively studied (Pintea *et al.*, 2001; Kallio *et al.*, 2000; Loskutova *et al.*, 1989; Stanescu *et al.*, 1989). In general, fatty acid composition of the oil from berries has been reported to be rich in palmitic and palmitoleic acids as well as in oleic, linoleic and linolenic acids (Cakir, 2004; Qibikeva, 1989). Our results are in agreement

Table 2. Fatty acid composition of seabuckthorn berries oil

Fatty Acid	Short hand designation	Sample name/amount (%)						
		Skardu red	Skardu yellow	Normal red	Bagrote yellow	Bagrote red	Shigar yellow	Shigar red
Myristic acid	$C_{14:0}$	0.31	0.21	0.21	----	0.21	0.21	0.11
Palmitic acid	$C_{16:0}$	29.76	28.85	28.53	36.52	17.83	33.23	17.62
Palmitoleic acid	$C_{16:1}$	26.60	25.67	24.31	32.41	13.24	29.12	12.41
Stearic acid	$C_{18:0}$	1.44	1.64	1.44	0.51	3.02	----	3.44
Oleic acid	$C_{18:1}$	22.25	22.79	22.14	22.63	30.03	21.30	37.07
Lenoleic acid	$C_{18:2}$	8.03	9.55	7.83	5.86	10.01	7.31	12.36
Lenolenic acid	$C_{18:3}$	0.30	0.21	0.21	0.10	----	0.10	0.73
Arachidic acid	$C_{20:0}$	0.31	0.31	0.14	0.10	0.28	0.10	1.42

with the reported values. Oil samples collected from Shigar were characterized by higher content of linoleic (12.36%), oleic (37.07%) and linolenic acids (0.73%) and the lowest percentage of palmitoleic acid (12.41%) as compared to other locations (Table 2). Yang and Kallio (2002; 2001) have reported similar observations. According to them, in the oil of whole berries, the proportion of palmitoleic acid correlates negatively with the proportion of linoleic and linolenic acids. It is clear from Table 2 that Bagrote oil sample has the highest percentage of palmitoleic acid (32.41%) and the lowest percentage of linolenic (0.1%) and linoleic acids (5.86%). These essential fatty acids play an important role in the prevention of heart diseases and cancer and improve overall immune system (Chai *et al*., 1989). Because of their effect on immunity, linoleic acid may have a useful role in treating disorders relating to hyper-stimulation of immune response (such as rheumatoid arthritis, psoriasis, multiple sclerosis, system lupus etc.).

The results showed that all samples were rich in high amounts of polyunsaturated fatty acids as compared to the saturated analogues.

Conclusion

Seabuckthorn is known as unique source of high valued oils. The oil of seabuckthorn has general nourishing, revitalizing, and restorative action. Its oil is a rich source of unsaturated fatty acids, phytosterols, carotenoids and flavonoides. Oil samples of seabuckthorn berries, collected from different altitudes of Skardu and Gilgit, have shown high concentration of palmitoleic acid, which differentiates it from most other oils of plant origin. In addition the oil of whole berries contains especially high level of caroteniods. The percentage of fatty acid varies depending on the area from where samples had been collected. All oil samples were rich in polyunsaturated fatty acids. The oil of berries collected from Shigar had high percentage of omega-3 and omega-6, while that from Bagrote area was high in palmitoleic acid. The results showed that concentration of polmitoleic acid and palmitic acid of pure pulp oil was greater than that of the saturated fatty acids. Density and refractive index are very stable parameters and may be used for checking the identity of oils. Triglyceride contents ensure the quality of oils. It is believed that imbalance in these vital essential fatty acids in our diet is the major reason for high incidence of heart diseases, hypertension, diabetes, obesity, colitis, premature aging and some types of Cancer (Eccleston *et al*., 2002).

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