

ANTIOXIDANT ACTIVITY IN EXTRACTS FROM SEA BUCKTHORN

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Abstract

The antioxidant components of sea buckthorne (*Hippophaë rhamnoides* L.) stored at 0.5, 10 and 20°C for 4 days were studied. Overall fruit quality declined more rapidly at 20°C. Weight loss of fruit was negligible for 2 days at all temperatures, it increased rapidly from day 3 at 20°C. Total phenolic compounds were slightly higher at 20°C than at other temperatures. Total ascorbic acid concentrations of the fruit remained similar for the first 2 days of storage, then declined in fruit stored at 0.5 and 20°C, but remained unchanged at 10°C. The total antioxidant activity of fruit was higher at 10°C than at 0.5 and 20°C on day 3. In conclusion, while the best temperature for long-term storage is 0.5°C, quality could be maintained at 10°C for acceptable periods of time for marketing and may be associated with better nutritional quality.

Key words: sea buckthorn, total phenols, antioxidant activity

INTRODUCTION

Sea buckthorn is a hardy plant, drought and cold tolerant, useful for land reclamation and farmstead protection [1]. The genus belongs to the family Elaeagnaceae, which consists of six species and 10 subspecies, among which the most economically important one is *Hippophaë rhamnoides* Linn., commonly known as sea buckthorn [2]. In summer, plentiful round yellow-orange berries cover the female plants. SB berries consist of a fairly tough skin and juicy pulp enveloping a small, hard, oval seed. Ripe berries from *H. rhamnoides* are orange-red and have a diameter of 10–15 mm and a soft outer fleshy tissue and hard seed. Soft parts of the berries, or pulp, contain 3–5% oil and seed contains 6–15% oil (1). SB berries are an excellent source of bioactive phytochemicals such as carotenoids, tocopherols, vitamin C, organic acids, and polyphenols [3].

Sea buckthorn (*Hippophaë rhamnoides* L.) is increasingly recognized as an important potential source of bioactive ingredients for functional foods, nutraceuticals, and personalcare products [4]. Sea buckthorn

(*Hippophaë rhamnoides*) fruits are of special interest because they are characterized by two different types of oil accumulation, which are characteristic for the mesocarp and seeds, respectively [5]. Sea buckthorn mesocarp oil includes not only the residues of widely occurring fatty acids, such as palmitic, stearic, oleic, linoleic, and linolenic, but also an unusual hexadecenoic (16:1) acid; in most cultivars of this plant species, this acid serves as a main fatty acid component of their oils [6]. Reverse-phase HPLC allowed detection of triglycerides (containing palmitoleic acid radicals) in sea buckthorne oil extracted from the parenchyma of the fruit on the background of the conventional triglycerides used as extractants. This allows rapid verification of the authenticity of the oil and detection of adulteration [7].

Sea buckthorn (*Hippophaë rhamnoides* L.) berries are a rich source of flavonoids, mainly flavonol glycosides and proanthocyanidins [8]. Sea buckthorn berries can be processed into jams, juices, yellow pigments, and seed oils [9]. Juice extraction from sea buckthorn berries leads to a residual press cake, which can be used for seed separation following extraction of seed oil. The remaining pulp is reported to contain mainly flavones [10]. Berries of sea buckthorn have a unique aroma not comparable to any

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The manuscript was received: 22.03.2013
Accepted for publication: 03.10.2013

other common fruit [11]. The flavor of sea buckthorn (*Hippophaë rhamnoides* L.) berries is perceived as sour and astringent mainly due to the high acid content, most importantly malic acid [12]. Due to the high acidity, the berry does not always attract consumers [13].

The plant has also been scientifically analyzed and reported to have antioxidative, immunomodulatory, cyto-protective, hepato-protective and tissue regenerative properties. The efficacy of sea buckthorn leaf extract, fruit and seed oils has been shown in dermalwound healing, burns of different etiology, skin radiation lesions, gastric and duodenal ulcers [14]. Moreover, sea buckthorn has demonstrated high ecological value; it has been planted on a large scale to combat soil erosion, particularly in China and Canada [15].

MATERIAL AND METHOD

Plant material:

Sea buckthorn (*Hippophaë rhamnoides* L.) berries were obtained from shrubs grown in Suceava county, România. The berries were cleaned manually to remove all foreign material and broken seeds. Sea buckthorn (*Hippophaë rhamnoides* L.) berries were divided in three groups and stored at 0.5, 10 and 20°C for 4 days.

Extraction procedure

Plant materials were sieved and 30 g were extracted with ethyl acetate in a Soxhlet apparatus. All the extracts were freeze-dried after evaporation in vacuum and stored at 0.5, 10 and 20°C. A further 30 g ground material was macerated with 200 mL of 70% aqueous methanol for 24 h and then filtered. This maceration procedure was repeated with fresh solvent for 5 days. All the macerates were combined and freeze-dried after removal of methanol and stored at 0.5, 10 and 20°C.

Weight loss determination

Weighed samples are placed in an oven for 4 days at 0.5, 10 and 20°C.

Determination of soluble solids concentrations

The soluble solid concentration was determined by refractometry.

Ascorbic acid determination

Determination of vitamin C content in sea buckthorn berries was cut by titration with 2,6-dichlorophenolindophenol (reagent Tillmans). The method is based on color change of the reagent, oxidation or reduction. Thus, the ionized form of 2,6-dichlorophenolindophenol is red in acid and blue in basic medium. Dehydroascorbic acid is obtained through reaction with vitamin C, and after reducing the identification reactive, 4-(p-hydroxyphenylamino)-2,6 dichlorophenol. This method is commonly used, due to the fact that it is easy to use and to the reagent sensitivity.

Total phenols

Total phenols were determined using the Folin-Ciocalteu reagent. 100 mL sample was transferred to a volumetric flask, to which 500 mL undiluted Folin-Ciocalteu reagent was subsequently added. After 1 min, 1.5 mL 20% (w v⁻¹) Na₂CO₃ was added and the volume made up to 10.0 mL with H₂O. After 2 h incubation at 25°C, the absorbance was measured at 760 nm and compared to a gallic acid calibration curve. Total phenols were determined as gallic acid equivalents.

2,2-Di (4-tert-octylphenyl)-1-picrylhydrazyl (DPPH) scavenging capacity assay

The method used for determining the antioxidant activity of sea buckthorn extracts is based on scavenging 2,2-Di (4-tert-octylphenyl)-1-picrylhydrazyl (DPPH) radicals. The decrease in absorbance was measured at 515 nm against a blank without extract, using a spectrophotometer. Using a calibration curve with different amounts of DPPH, the IC₅₀ was calculated. The IC₅₀ is the concentration of an antioxidant that is required to quench 50% of the initial DPPH radicals under the experimental conditions given.

Statistics

Samples were assayed in triplicate and results are given as averages ± SD. Student's t test was used for the statistical evaluation and *P* < 0.05 was considered statistically significant.

RESULTS AND DISCUSSIONS

Weight loss determination

In Table 1 weight loss values for sea buckthorn berries samples at temperatures of 0.5, 10 and 20°C are presented.

As seen from the data presented in Table 1, weight loss of fruit was negligible for 2 days at all temperatures it increased rapidly from day 3 at 20°C.

Table 1 Weight loss of sea buckthorn berries

	Weight loss (%)			
	1 day	2 day	3 day	4 day
0.5°C	0.45 ± 0.01	0.43 ± 0.02	0.72 ± 0.1	1.13 ± 0.11
10°C	0.55 ± 0.2	0.65 ± 0.01	2.11 ± 0.02	3.84 ± 0.01
20°C	0.64 ± 0.02	0.84 ± 0.1	5.22 ± 0.01	9.62 ± 0.02

Ascorbic acid determination

Ascorbic acid concentrations of the fruit remained similar for the first 2 days of storage, then they declined in fruit stored at 0.5 and 20°C, but remained unchanged at 10°C (Figure 1).

The variation of ascorbic acid throughout the storage period (Figure 1) showed that the vitamin content can be affected by temperature. Storage at 0.5°C and 10°C temperature was an effective way to maintain the initial level of total ascorbic acid for additional days, but it did not increase the vitamin content significantly.

There are reports [16] of the positive influence of low temperature in the maintenance of vitamin C content during storage of fruit and vegetables.

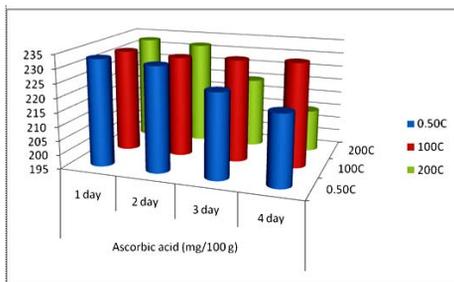


Figure 1 Ascorbic acid concentrations of sea buckthorn berries (■ - 0.5°C; ■ - 10°C; ■ - 20°C)

Total phenols

Total phenols content of sea buckthorn berries is not altered by storage (Figure 2). The total phenols content was assayed using the Folin-Ciocalteu reagent method and is expressed as $\text{g}_{\text{gallic acid}} \text{kg}^{-1}_{\text{extract}}$.

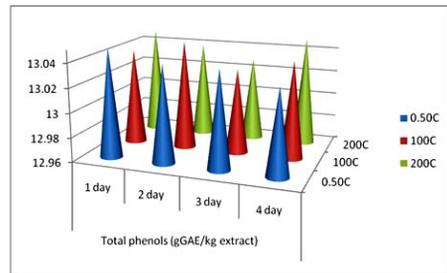


Figure 2 Total phenols of sea buckthorn berries (■ - 0.5°C; ■ - 10°C; ■ - 20°C)

2,2-Di (4-tert-octylphenyl)-1-pyrcilhydrazyl (DPPH) scavenging capacity assay

In Figure 3 the IC₅₀ values are indicated. The lowest IC₅₀ value indicates the highest free radical scavenging activities. Free radicals are involved in the process of lipid peroxidation and are considered to play a cardinal role in numerous chronic pathologies, such as cancer and cardiovascular diseases among others, and are involved in the aging process. Therefore, the extracts were assessed against DPPH radicals to determine their free radical scavenging properties. In this assay, the DPPH radical serves as the oxidizing substrate, which can be reduced by an antioxidant compound to its hydrazine derivative via hydrogen donation, and as the reaction indicator molecule.

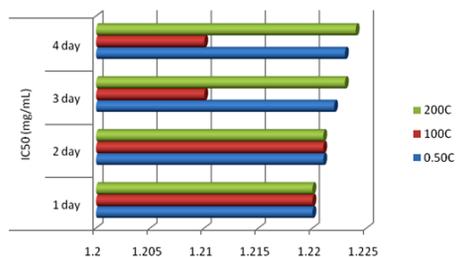


Figure 3 Free Radical Scavenging Activities of extract of sea buckthorn berries (■ - 0.5°C; ■ - 10°C; ■ - 20°C)

The total antioxidant activity of fruit was higher at 10°C than at 0.5 and 20°C on day 3.

CONCLUSIONS

Sea buckthorn has various bioactive components with potential health benefits, including anti-diabetic, anti-obese, anti-oxidant and anti-inflammatory.

In conclusion, the data presented in this paper indicated that the storage temperatures affect the antioxidant capacity of sea buckthorn berries. The content of total phenolics was not affected by the temperature lowering, while ascorbic acid was affected by temperature. The total antioxidant activity of fruit was higher at 10°C than at 0.5 and 20°C on day 3. The free radical scavenging property may be one of the mechanisms by which this drug is useful as health food.

While the best temperature for long-term storage is 0.5°C, quality could be maintained at 10°C for acceptable periods of time for marketing and may be associated with better nutritional quality. This finding is not only of sensorial or health relevance, but also economical, because of the additional costs involved in the maintenance of lower temperatures.

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