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ORIGINAL PAPER

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Changes in sugar, organic acid, flavonol and carotenoid composition during ripening of berries of three seabuckthorn (*Hippophae rhamnoides* L.) cultivars

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Abstract Soluble solid, sugar, organic acid, flavonol and carotenoid content were determined in seabuckthorn berries of the three German cultivars Askola, Hergo and Leikora, collected at different harvesting times, to provide a more thorough knowledge of quality changes occurring during ripening of the berries. The main organic acids were malic (1940–4660 mg/100 g), quinic (810–2820 mg/100 g), ascorbic (180–370 mg/100 g) and citric acid (90–160 mg/100 g). In all three cultivars a marked decline in total organic acid concentration was observed during ripening. The pattern of variation of sugars, glucose (0.26–2.10 g/100 g) and fructose (0.14–0.54 g/100 g), was somewhat different among the three cultivars. In all three cultivars ascorbic acid concentration decreased during ripening. The main flavonols were isorhamnetin (350–660 mg/kg), quercetin (30–100 mg/kg) and kaempferol (2–5 mg/kg). The trends of flavonol content during ripening were quite different among the three cultivars. The main carotenoids were zeaxanthin (30–150 mg/kg), β-carotene (3–50 mg/kg) and β-criptoxanthin (5–19 mg/kg). The genotype seemed to affect both the extent of carotenoid accumulation and the carotenoid profile but in all three cultivars ripening was accompanied by an increase in total carotenoid concentration. The various classes of antioxidants showed quite different patterns of variation during ripening, achieving their maximum level at different harvesting dates.

Keywords Seabuckthorn · *Hippophae rhamnoides L* · Cultivar · Ripening · Sugars · Organic acids · Ascorbic acid · Flavonoids · Carotenoids

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Introduction

Seabuckthorn (*Hippophae rhamnoides* L.) is a berry-bearing bush of the family *Elaeagnaceae*, naturally distributed in Asia and Europe [1]. Since ancient times seabuckthorn berries have been used as table fruits, raw material for juices and medicinal ingredient in Asian countries. More recently, the clinical use of many medicinal preparations of seabuckthorn for the treatment of a range of diseases has spread in China and in the former Soviet Republics [2].

At present, various food products are derived from seabuckthorn berries, ranging from juices to jams and food additives for jellies and candies [3]; moreover, seabuckthorn oils, extracted from both pulp and seed, are marketed as food ingredients and supplements. Gelatine or vegetable-based capsules and oral liquid are targeted especially for maintaining the health of skin, mucosa, cardiovascular and immune systems [2].

The soft parts of the berry (fruit flesh and peel) are particularly abundant in bioactive compounds, such as ascorbic acid (300–25,000 mg/kg fresh berries) [4], phenolic compounds (1,100–2,100 mg/kg) [5], tocopherols and tocotrienols (10–150 mg/kg) [2], carotenoids (30–150 mg/kg) [5] and sterols (350–500 mg/kg) [6]. The biological action of these minor compounds, together with that elicited by fatty acids, has been supposed to contribute to the various beneficial physiological effects associated with ingestion of seabuckthorn pulp oil and juice. Oral administration of seabuckthorn berry juice increased the plasma HDL-cholesterol concentration and decreased the susceptibility of LDL to oxidation in humans [7]. Supplementation of pulp oil significantly improved symptoms in atopic dermatitis patients and increased the level of plasma HDL-cholesterol [8]. Oral administration of pulp oil to healthy normolipidemic men inhibited platelet aggregation [9], whereas a flavonoid extract from seabuckthorn berries showed in vivo antithrombotic properties in an animal model, probably due to inhibition of platelet aggregation [10]. Moreover, in vitro assays evidenced marked cytoprotective properties of

seabuckthorn berry extracts, which could be attributed to their anti-oxidant activity [11]. All these findings corroborate the image of seabuckthorn berry as a candidate for a functional food ingredient. For example, seabuckthorn juice has been proposed as a convenient ingredient for fruit juices with special antioxidative properties [7].

Consequently, the level of these bioactive compounds represents an important quality parameter of the berries. Recent studies have thoroughly investigated the effects of genotype, harvesting time and climatic conditions on the content of vitamin C, tocopherols and phytosterols in seabuckthorn berries [6, 12, 13]. On the other hand, few reports have dealt with the compositional profile of other two important classes of seabuckthorn berry antioxidants, phenolic compounds [14] and carotenoids, and, in particular, few and incomplete data are available on their changes during ripening [5, 15].

Moreover, when considering the potential food application of seabuckthorn berries, it is important to take into account other chemical parameters such as the compositional profile in organic acids and sugars, which play a key role in determining the sensory properties and, eventually, consumer acceptability of seabuckthorn products. Tang and colleagues [16] found that titratable acidity was positively correlated with the intensity of astringency, sourness and, negatively, with sweetness of seabuckthorn juice, whereas the sugar:acid ratio was associated with juice pleasantness.

In this study we determined some chemical parameters (soluble solids, sugars, organic acids including ascorbic acid, flavonols and carotenoids) on the soft part of seabuckthorn berries of the three German cultivars Askola, Hergo and Leikora, collected at different harvesting dates. The three selected cultivars are already known to show a good adaptation to the warm climate of central Italy. The aim of the present study was to provide a more thorough knowledge of compositional and quality changes that occur during ripening of seabuckthorn berries, and to contribute to a better definition of the optimal harvesting date in relation to the final destination of the product.

Materials and methods

Sampling

Berries of the three cultivars of *Hippophae rhamnoides*, Askola, Hergo and Leikora, were collected from mature trees (5–7 years old) grown in an experimental field of the Fruit Tree Research Institute near Rome (latitude 41°48' N, longitude 12°35' E, and altitude 117 m). Since the season is normally dry throughout the entire summertime, plants were drip irrigated from the end of May until mid-September to avoid interference from water stress. Fruits were harvested from early July (all cultivars unripe) to the end of August (for Leikora) or September (for Askola and Hergo). At each date, approximately 75 berries, collected from 5 different branches, were processed immediately for individual fruit weight, soluble solids content and juice pH determination, while the rest of the sample was stored at –20 °C until analysis. Soluble solids were determined by a Bausch and Lomb table refractometer and expressed as Brix degrees.

Biochemical analyses

All chromatographic analyses were performed on a Hewlett Packard (Palo Alto, CA) HPLC system with a 1100 series quaternary pump and a diode array UV-vis detector.

Organic acids and ascorbic acid

Berry samples (5 g) were extracted with 2% metaphosphoric acid (100 mL) by homogenising and then stirring for 10 min. After centrifugation (at 20,000×*g* for 10 min at 4 °C), the aqueous layer was filtered through a 0.2 m cellulose syringe filter and injected. The chromatographic column was a Inertsil 5 ODS-2 (ChromSep Varian) (5 μm, 4.6×250 mm), with a guard column ChromSep SS (5 μm, 4.6×10 mm), and was thermostatted to 35 °C. The organic acids were eluted isocratically with 40 mM KH_2PO_4 (pH 2.8) at a flow rate of 1 mL/min and the eluate was monitored at 214 nm. Quantification was achieved by calibration curves obtained with authentic standards (Sigma).

Flavonols

Flavonols were determined as aglycones after hydrolysis. Extraction and hydrolysis were carried out as described by Hakkinen and Auriola [17]. Homogenised berry samples (5 g) were refluxed for 2 h at 85 °C in 50% (v/v) aqueous methanol containing hydrochloric acid (1.2 M) and *tert*-butylhydroquinone (Sigma) as an antioxidant. The chromatographic column was a Supelcosil LC-18 (5 μm, 4.6×250 mm), with a guard column Supelcosil LC-18 (5 μm, 4.6×10 mm), and was thermostated to 30 °C. Solvent A was 1% formic acid, and solvent B was acetonitrile. The gradient used was: 0 min 90% of A, 10% of B; 10 min 80% of A, 20% of B; 32 min 60% of A, 40% of B; 35 min 30% of A, 70% of B; 40 min 30% of A, 70% of B; 42–50 min 90% of A, 10% of B. Flow rate was 1 mL/min and volume injected 20 μL. Peaks were detected at 370 nm. Quantification was achieved by a calibration curve obtained with authentic standard (quercetin and kaempferol from Sigma, isorhamnetin from Fluka).

Carotenoids

The extraction and saponification procedure described by Hart and Scott [18] was followed with slight modifications. Berry samples (5 g) were extracted in tetrahydrofuran and methanol (1:1 v/v THF:MeOH), containing 0.1% butylated hydrohytoluene (BHT) (Sigma) as an antioxidant. $CaCO_3$ (0.5 g), to neutralise acids, and β-apo-8'-carotenal (Fluka), as an internal standard, were added. Carotenoids were extracted by homogenising for 3 min using an ultra-turrax homogeniser at 0 °C; this step was repeated four times. After filtration the THF/MeOH solution was extracted by adding petroleum ether (40–60% fraction, containing 0.1% BHT) and a saturated solution of NaCl, and mixing by careful shaking. The upper petroleum ether phase was transferred to an evaporating flask, whereas the THF/MeOH/aqueous phase was extracted two more times with petroleum ether. The combined petroleum ether phases were evaporated at 35 °C to near dryness and then re-dissolved in 10 mL dichloromethane (DCM) containing 0.1% BHT. The DCM extract was saponified with an equal volume of 10% potassium hydroxide in MeOH (under nitrogen, in the dark) for 1 h at room temperature. After saponification the carotenoids were extracted from the KOH/MeOH phase by shaking with petroleum ether and a saturated solution of NaCl. The KOH/MeOH/aqueous phase was extracted two more times with petroleum ether and the petroleum ether phases combined and washed with water until washings were neutral to pH paper. The petroleum ether phases were evaporated at 35 °C to near dryness and re-dissolved in 40% acetonitrile, 20% methanol, 20% hexane, 20% dichloromethane and filtered through a 0.2 μm cellulose syringe filter. The chromatographic column was a Supelcosil LC-18 (5 μm, 4.6×250 mm),

with a guard column Supelcosil LC-18 (5 μm, 4.6×10 mm), and was thermostated to 30 °C. Carotenoid elution was achieved by using the following linear gradient: 0–10 min 95% A, 5% B; 20 min 80% A, 20% B; 40 min 66% A, 34% B; 45 min 58% A, 42% B; 50 min 95% A, 5% B, where A was acetonitrile and B methanol/hexane/dichloromethane 1:1:1 v/v. Flow rate was 0.8 mL/min and volume injected 20 μL. Peak responses were measured at 450 nm. Quantification of carotenoids was achieved by calibration curve obtained with authentic standard (β-carotene from Fluka, zeaxanthin and β-cryptoxanthin from Extrasynthese).

Sugars

Glucose and fructose were determined in duplicate using an enzyme-assay kit, according to the guide for test-kits for D-Glucose/D-Fructose (Scil Diagnostic, Martinsried, Germany). Samples (10 g of frozen berries) were crushed using an ultra-turrax homogeniser, made up to 200 mL with re-distilled water and then stirred for 10 min. After acidity was adjusted to approximately pH 7 with KOH 2 N, the solution was centrifuged (10,000 rpm for 10 min) and the aqueous phase filtered and used for the enzymatic assay. The absorbance measurements were carried out on a Kontron spectrophotometer (Kontron, Unikon 930, Watford, UK).

Results and discussion

Repeated observations, conducted over several years in the same experimental field, confirmed that ripening of the berries began at approximately mid-July and went on till September. In some cases, however, after the end of August some berries started shrinking, indicating that over-ripeness was approaching; this was observed, in particular, for Leikora and for this reason we did not consider any date later than the end of August for this cultivar. Figure 1 shows the entire berry growth period, just for an extensive evaluation of the trend, but our study on compositional changes was focused only on the ripening period.

Leikora was characterised by the largest berries (mean berry weight equalled 450–550 mg, during ripening) when compared to Askola and Hergo (200–380 mg and 150–320 mg, respectively). One should note the high variability among the berries on the same branch, or even on the same spur, despite the fact that they were always sampled from well lit branches, at the periphery of the canopy. The same high variability among the berries on the same spur or branch was also detected when measuring the (static) detachment force, which ranged from 50 to 500 g and over (data not tabulated). The curve of the berry weight increase showed some differences among the three cultivars: in Leikora, berry weight increased until around the beginning of August and then remained practically constant, showing a pattern similar to that observed by Tang [13] on seven seabuckthorn genotypes grown in Finland, but shifted approximately one month earlier. In contrast, we observed a nearly constant rate of increase, from early stages of maturation until the end of September, in Askola and a similar trend in Hergo, except for a short interruption at the beginning of August. Differences between cultivars were also observed in the changes in the content of soluble solids during the ripening period (Fig. 2): Leikora maintained approximately constant levels throughout the period (about 7.5°Brix), Askola showed a slight decline (from 11.2 to 8.8°Brix), whereas Hergo was characterised by a marked reduction (from about 12.8 to 7.5°Brix).

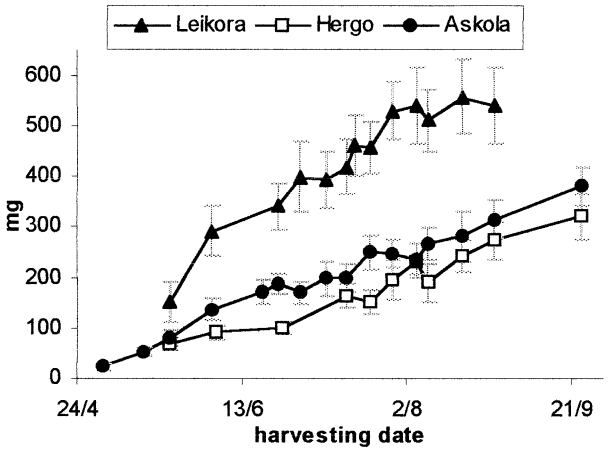

Fig. 1 Berry weight (mg) increase with time

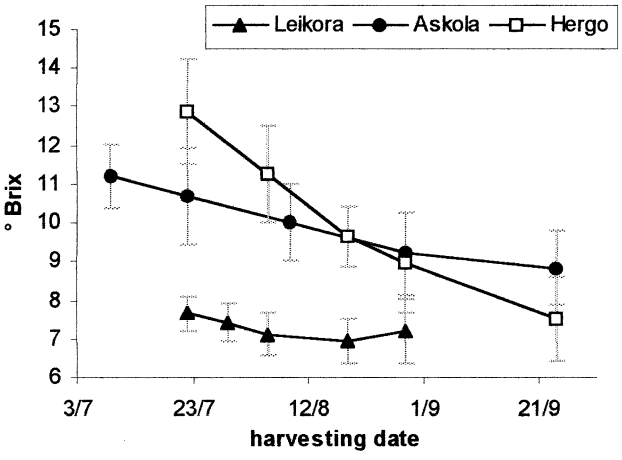

Fig. 2 Soluble solids content (°Brix) at different harvesting dates

Organic acids and sugars

Organic acids and sugars constitute the main part of the soluble solid fraction of the soft parts of seabuckthorn berry. In agreement with the significant decrease in titratable acidity observed by Tang [13], in all three cultivars total organic acid concentration showed a marked decline during ripening (Fig. 3): from 7,500 to 5,830 mg/100 g in Askola, from 5,600 to 4,250 mg/100 g in Hergo and from 4,090 to 3,430 mg/100 g in Leikora. This reduction reflected a dilution effect, due to the water uptake occurring during the berry expansion showed in Fig. 1; really, the total amount of organic acids in 100 berries gradually increased in Askola (from about 1,500 to 2,200 mg/100 berries) and in Hergo (from 850 to 1,350 mg/100 berries), whereas it did not show a definite

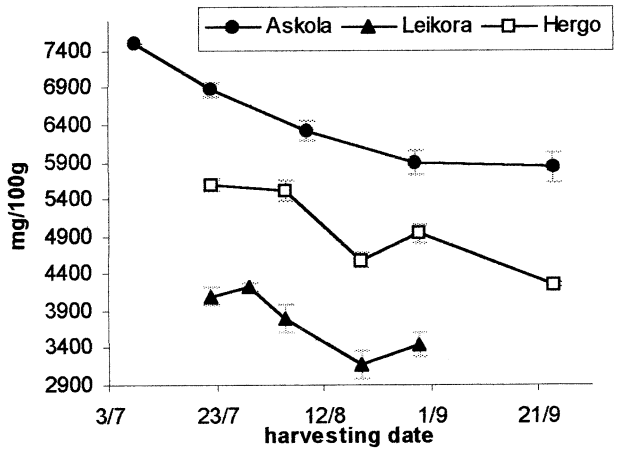

Fig. 3 Total organic acids content (mg/100 g berry fresh weight) at different harvesting dates

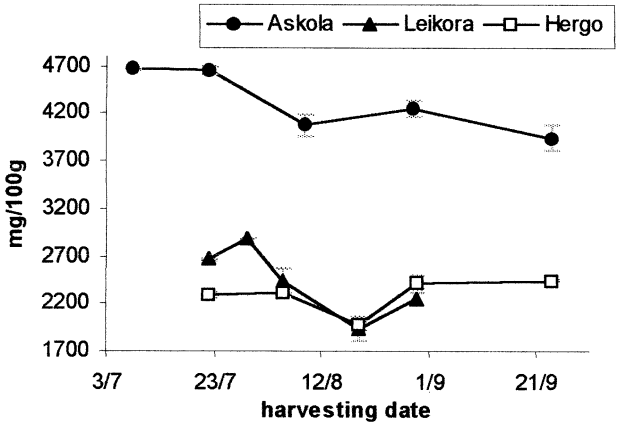

Fig. 4 Malic acid content (mg/100 g berry fresh weight) at different harvesting dates

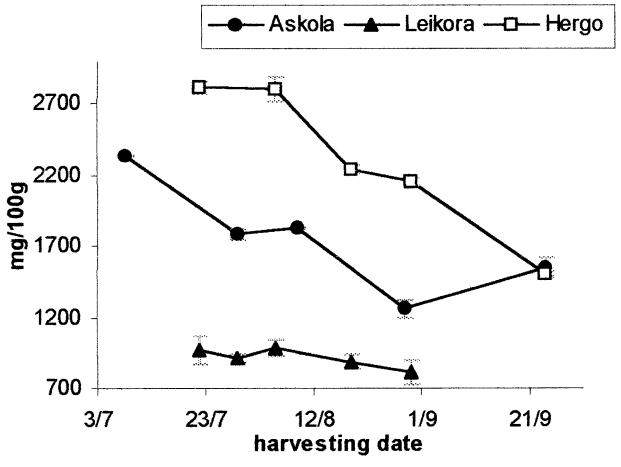

Fig. 5 Quinic acid content (mg/100 g berry fresh weight) at different harvesting dates

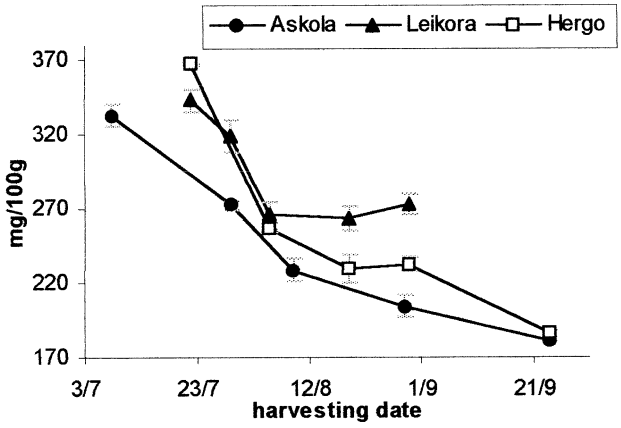

Fig. 6 Ascorbic acid content (mg/100 g berry fresh weight) at different harvesting dates

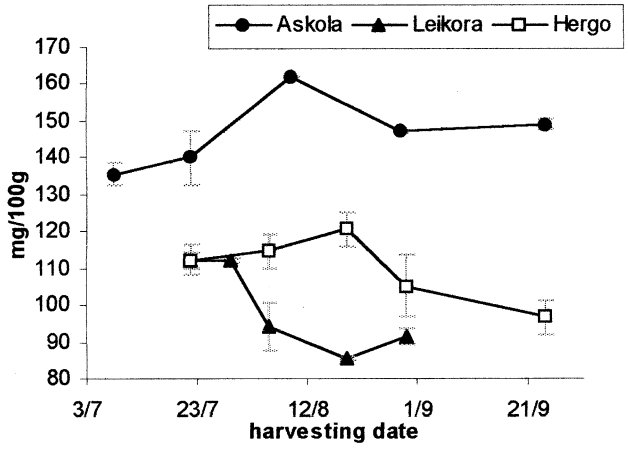

Fig. 7 Citric acid content (mg/100 g berry fresh weight) at different harvesting dates

trend in Leikora (1,750–2,250 mg/100 berries). Moreover, juice pH ranged between 2 and 2.5 in all cultivars and showed no significant variations during the ripening period (graph not shown).

In all cultivars the main organic acids were malic (Fig. 4), quinic (Fig. 5), ascorbic (Fig. 6) and citric acid (Fig. 7). Askola and Leikora showed a similar organic acid profile (malic acid about 65% of total acids, and quinic acid about 25%); Hergo was characterised by a lower malic:quinic acid ratio, which, on the other hand, tended to increase during ripening (malic acid varied from 40% to 57% of total acids, whereas quinic acid decreased from 50% to 35%). Malic (particularly in Askola and in Leikora) and quinic acid (in Askola and in Hergo) mainly contributed to the global decrease of total organic acid content.

Glucose and fructose constitute nearly the whole sugar fraction of seabuckthorn berries [3, 13]. Until the end of August Hergo showed a significantly higher sugar content than the other cultivars (2.26–2.64 g/100 g versus 0.98–1.62 g/100 g in Askola and 0.40–0.64 g/100 g in Leikora)

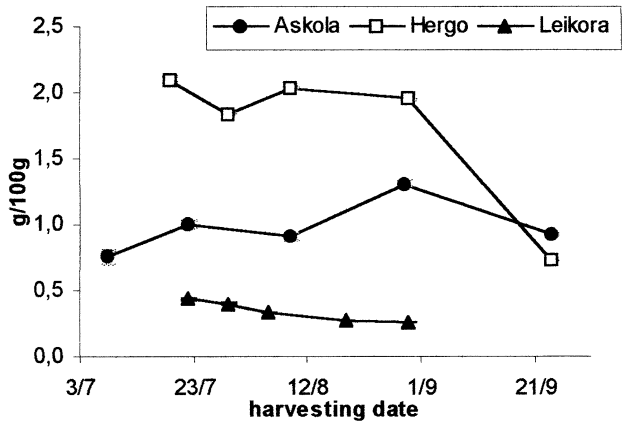

Fig. 8 Glucose content (g/100 g berry fresh weight) at different harvesting dates

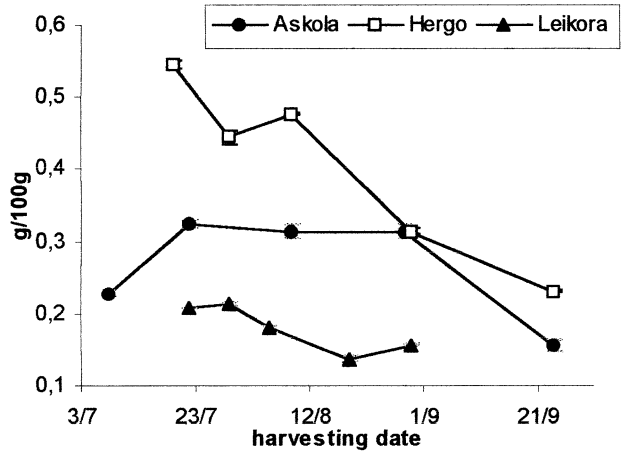

Fig. 9 Fructose content (g/100 g berry fresh weight) at different harvesting dates

(Figs. 8 and 9); this, combined with an intermediate level of organic acids, contributed to a higher sugar:acid ratio in Hergo (0.41–0.55 versus 0.13–0.27 in Askola and 0.12–0.16 in Leikora), which could reflect in a higher organoleptic quality. In all cultivars glucose was the major sugar component and the glucose:fructose ratio was relatively constant during ripening period (Askola 2.9–5.9, Hergo 3.2–6.2 and Leikora 1.7–2.1). Trends in sugars content were somewhat different among the three cultivars: in Hergo sugar content remained relatively high till the end of August and then showed a sharp decrease; in Askola it increased up to a peak at the end of August and then declined; in Leikora it showed a slowly declining trend. A marked variability in sugar accumulation pattern among different genotypes were previously observed by Tang [13].

The sugar:acid ratio has been reported to constitute the major promoter of taste of seabuckthorn berry juice [16]. In our experiment we observed its highest values in berries harvested from the middle to end of August for Askola and Hergo, whereas in Leikora it slightly declined throughout the harvesting period.

Ascorbic acid

Various investigations have shown considerably variable levels of vitamin C in seabuckthorn berries of different origin. It was observed that genetic background is the most important factor in determining the vitamin C content, and secondly, that the degree of ripeness affects it more than other factors such as climatic conditions [4, 12]. Figure 6 presents the ascorbic acid content we determined in berries harvested at different times: in early August (Askola 229 mg/100 g, Hergo 257 mg/100 g, Leikora 266 mg/100 g) it was higher than that observed in seabuckthorn selections of Finnish origin (subsp. *rhamnoides*) (mean values around 130 mg/100 g) [4], and in Russian cultivars (65–176 mg/100 g) [5, 15], all grown in North Europe, but lower than those of Chinese origins (subsp. *sinensis*) (mean values 460 mg/100 g) [4]. Similar differences associated with the origin of seabuckthorn populations were also observed by Kallio and colleagues [12]: juice obtained from berries native to China was markedly higher in vitamin C content (400–1,300 mg/100 mL) than that from berries from North Europe and Russia (2–200 mg/100 mL). On the other hand, the levels observed in our work were close to those determined by Gatke and colleagues [19] in berries of the same cultivars, Hergo and Leikora (mean value 266 mg/100 g), grown in Germany. The conformity between values for these German cultivars observed out of their native environment with their corresponding values in native environment seemed to confirm the high genetic stability for this biochemical trait, as already observed [4].

In all three cultivars results showed a clear decreasing trend in ascorbic acid concentration during ripening. In Askola and Hergo the level gradually declined throughout the considered period, whereas in Leikora it rapidly decreased until early August, and remained practically constant afterwards. Accordingly, in all cultivars the maximum levels were observed at the first time point (early or mid-July). In Askola ascorbic acid content varied from 333 to 181 mg/100 g (with a rate of variation of −1.94 mg/day), in Hergo from 367 to 186 mg/100 g (−2.83 mg/day), in Leikora from 343 to 273 mg/100 g (−1.79 mg/day). Similar behaviour was observed in other cultivars grown in a colder climate: in five Russian cultivars, grown in Finland, Jeppsson and colleagues [15] observed a mean reduction from 148 to 110 mg/100 g (−2.00 mg/day) and in three Russian cultivars, grown in Sweden, Gao and colleagues [5] determine a mean reduction from 107 to 76 mg/100 g (−1.64 mg/day). Similar trends were also observed in four cultivars derived from the subspecies *rhamnoides* and grown in Finland [12]. On the other hand, in all these cases, fruit ripening was accomplished later in the season (from August to November) relative to our experiment, and accordingly the time at which ascorbic acid reached the highest level was shifted later (early to late August) [5, 12, 20].

It is interesting to note that the reduction of ascorbic acid concentration was due to the above mentioned dilution effect. As already observed by Tang [13], the

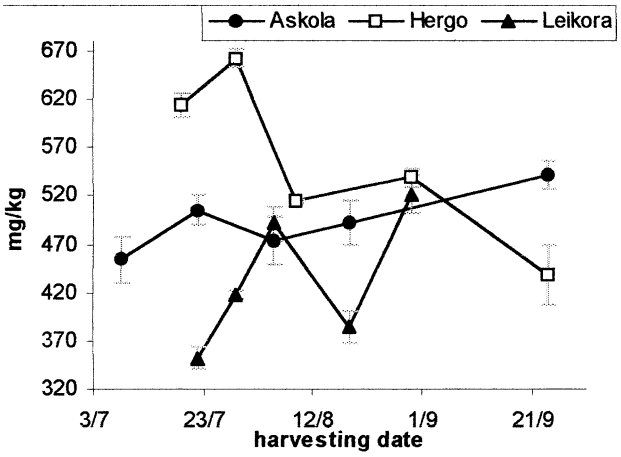

Fig. 10 Isorhamnetin content (mg/kg berry fresh weight) at different harvesting dates

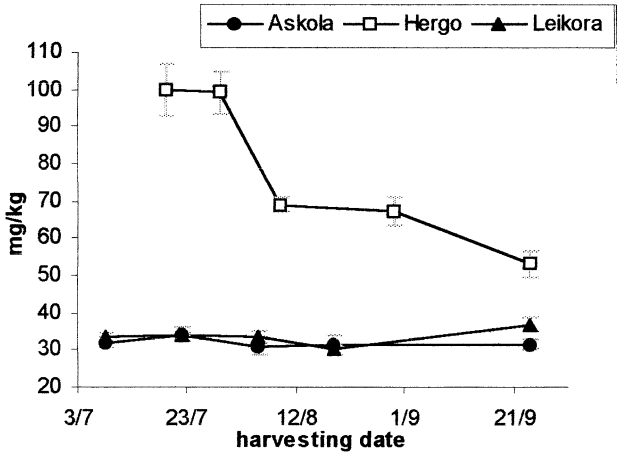

Fig. 11 Quercetin content (mg/kg berry fresh weight) at different harvesting dates

amount of ascorbic acid in 100 berries was nearly constant during ripening (in Hergo it ranged from 55.5 to 63.1 mg/100 berries, in Askola from 60.8 to 67.6 mg/100 berries, in Leikora from 143.0 to 168.5 mg/100 berries).

Flavonols

As recently reported flavonols, together with proanthocyanidins, represent the main class of phenolic compounds present in seabuckthorn berries [14]. Isorhamnetin (Fig. 10) was by far the main flavonol in all the three cultivars examined, whereas quercetin was present at lower level (Fig. 11), in agreement with data previously reported on seabuckthorn juice [7, 14]. Some of the main isorhamnetin and quercetin glycosides detected in seabuckthorn berry juice have been recently identified by Rosch and colleagues [14]. Isorhamnetin concentration was particularly high in the early stages of ripening in Hergo (614 and 662 mg/kg), and relatively low in the first and fourth time points (mid-July and mid-August) in Leikora (351 and 384 mg/kg). In all other cases, we found similar isorhamnetin levels in the three cultivars (Askola 454–541 mg/kg, Leikora 417–521 mg/kg, Hergo 438–538 mg/kg). Secondly, at all ripening stages, Hergo was characterised by significantly higher content of quercetin with respect to the other two cultivars (53–100 mg/kg versus 31–34 mg/kg in Askola and 30–37 mg/kg in Leikora). Moreover we detected relatively low levels of kaempferol: 4.0–5.3 mg/kg in Hergo, 1.8–2.1 mg/kg in Askola and 2.7–3.2 mg/kg in Leikora (data not shown).

In the chromatograms we also observed an unidentified peak, whose area was similar in magnitude to that of quercetin peak in Leikora and was markedly lower in the other two cultivars. The similarity in retention time and spectral characteristics of the unidentified peak to those of myricetin suggested that the unknown compound could be an isomer of myricetin with a different hydroxylation pattern in the B-ring.

The trends of flavonol content during ripening were quite different among the three cultivars. In Hergo the first two time points (mid- and late July) were characterised by isorhamnetin and quercetin levels markedly higher than the following dates; from the end of July to the end of September the reduction equalled –33% for isorhamnetin and –47% for quercetin. In Askola isorhamnetin content showed a slight increase from early July to late September (+19%), whereas no significant changes were observed in quercetin level. In Leikora isorhamnetin content showed significant variations between different dates, but did not evidence a definite changing trend, whereas, similarly to Askola, quercetin levels were practically constant throughout ripening.

The only paper, to our knowledge, reporting on flavonol changes during ripening of seabuckthorn berry is that by Jeppsson and colleagues [15]: they determined quercetin and kaempferol, but not isorhamnetin, in three Russian cultivars (Otradnaja, Prozratnaja and Gibrid Pertjik) grown in Finland. For 19 days around ripening, a significant reduction in quercetin levels was observed only in Otradnaja (from 28 to 14 mg/kg), whereas no significant changes were revealed in the other two cultivars; moreover, a slight increase was observed in kaempferol content in both Prozratnaja and Gibrid Pertjik (from 12 to 16 mg/kg). In most fruits (apples, red currants, sour cherries, plums and blueberries) total flavonol content decreases during fruit maturation, but there are a few exceptions (blackcurrant, grape) in which the opposite trend has been observed [21]. Furthermore, actual flavonol concentration in fruit tissues can be significantly affected by various contingent biotic and abiotic stresses, like pathogen attack, wounds or high UV irradiation, which can induce their biosynthesis [22].

It is interesting to note that the ratio isorhamnetin: quercetin was also significantly different in the three cultivars: during ripening it increased linearly in Hergo (from 6.1 to 8.3) and in Askola (from 14.2 to 17.2), whereas in Leikora it wasin the range 10.5–14.6. The value calculated for this ratio (6.3) from isorhamnetin and quercetin content determined by Rosch and colleagues

[14] in a juice obtained from berries of Hergo, substantially agreed with the range that we calculated for the same cultivar. In contrast, in the same juice they found a markedly higher proportion of kaempferol than in our berry samples (11.3% kaempferol percent of total flavonols versus 0.6–0.9%).

Total seabuckthorn berry flavonol content (415–765 mg/kg) was confirmed to be particularly high if compared to other common vegetables and fruits (generally below 20 mg/kg) [23], and edible berries (up to 270 mg/kg) [24]. Total flavonol content in seabuckthorn juices have been reported to equal 310–350 mg/L [7, 14], whereas in common fruit juices it is generally below 5 mg/L [25], so that the addition of seabuckthorn juice to common fruit juices could markedly increase their flavonol content. In general, the consumption of seabuckthorn products could significantly contribute to the dietary intakes of flavonols, which has been estimated in the Seven Countries Study to vary in the range 6–60 mg/day [25].

Eccleston and colleagues [7] suggested that the high level of flavonoids, together with ascorbic acid, ingested with seabuckthorn juice could contribute in vivo to an improved LDL oxidation status. Moreover, in a recent study on an animal model, a flavonoid seabuckthorn extract has shown an effect on in vivo thrombogenesis similar to that of aspirin: this effect could be partly attributed to the synergistic action of quercetin and catechins (present in polymeric form in proanthocyanidins, the other main phenolic class in seabuckthorn) in suppressing platelet aggregation [10].

The seabuckthorn flavonol profile was characterised by the high proportion of isorhamnetin, which is not very common in fruit and generally represents a minor constituent of flavonol fraction. In in vitro assays, isorhamnetin has been reported to show a low free radical scavenging capacity and inhibition effect on peroxidation in human LDL [14, 26]. Nevertheless, studies on rats have shown that isorhamnetin was the main metabolite of quercetin found in plasma, and that at least part of the in vivo beneficial effects of quercetin may result from its conversion to isorhamnetin [27]. In another study on rats, orally administrated isorhamnetin diglucoside has been shown to be metabolised in vivo by intestinal bacteria to its aglycone, and isorhamnetin was found to be effective as an antioxidant [28]. In any case, a clear elucidation of the pharmacodynamics of isorhamnetin glycosides is required to evaluate their possible biological effects in humans.

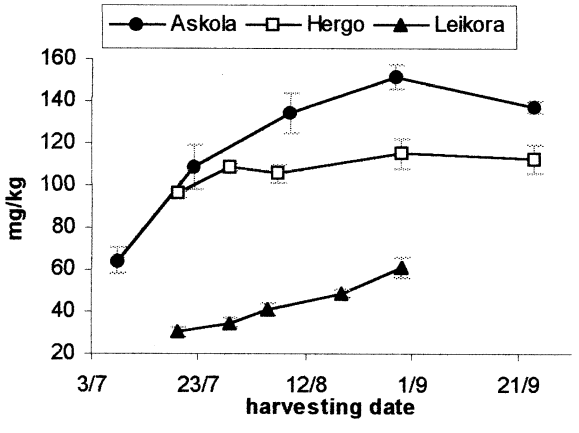

Fig. 12 Zeaxanthin content (mg/kg berry fresh weight) at different harvesting dates

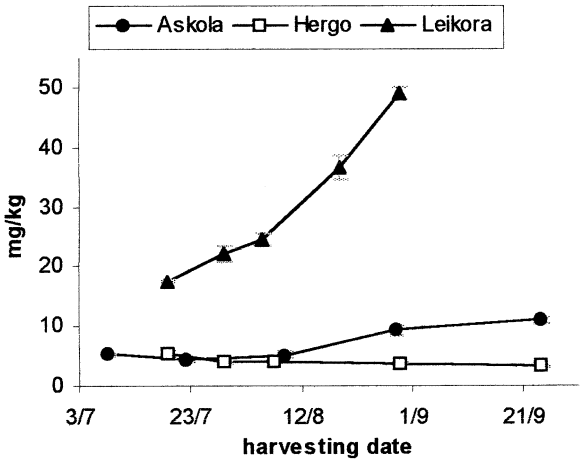

Fig. 13 β-Carotene content (mg/kg berry fresh weight) at different harvesting dates

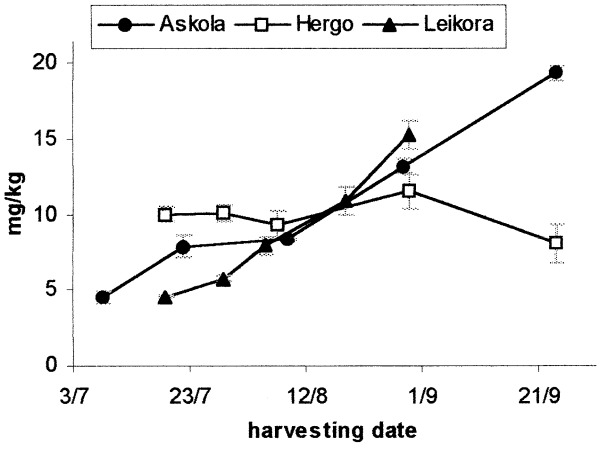

Fig. 14 β-Criptoxanthin content (mg/kg berry fresh weight) at different harvesting dates

Carotenoids

In all three cultivars the main carotenoids were zeaxanthin (Fig. 12), β-carotene (Fig. 13) and β-criptoxanthin (Fig. 14), which were the principal pigments of the bright orange-coloured berries. The sum of the three carotenoids amounted to 74–174 mg/kg in Askola, 111–130 mg/kg in Hergo and 52–125 mg/kg in Leikora. The carotenoid profile was similar in Askola and Hergo: zeaxanthin constituted 82–91% of the sum of the three main carotenoids in Askola (64–151 mg/kg) and 86–91% in Hergo

(96–115 mg/kg), whereas β-carotene represented 3.5–7.1% in Askola (4.4–10.9 mg/kg) and 2.6–4.8% in Hergo (3.2–5.4 mg/kg). On the other hand, Leikora showed a quite different profile, with relatively lower levels of zeaxanthin (30–61 mg/kg, equal to 49–57% of the sum) and higher content of β-carotene (18–49 mg/kg, equal to 33–39% of the sum). β-Criptoxanthin levels were similar in the three cultivars (4.5–19.3 mg/kg in Askola, 8.1–11.5 mg/kg in Hergo, 4.6–15.2 mg/kg in Leikora). The total content of carotenoids has been reported to be subject to extreme variation, with differences up to tenfold even within the same natural population and subspecies: Yang [2] reported a quite large range for total carotenoids (from 10 to 1,200 mg/kg) and β-carotene (from 2 to 170 mg/kg), whereas Beveridge and colleagues [3] quoted common levels for total carotenoids ranging from 20 to 345 mg/kg. Few data have been reported in the literature regarding the carotenoid profile. According to Yang [2], β-carotene constitutes 15–55% of total carotenoids, depending on the origin, and some papers on Russian cultivars reported proportions within that range (16–44%) [29, 30]. In contrast, our data showed that the two cultivars Askola and Hergo were characterised by quite a low proportion of β-carotene. Moreover, in the Russian variety Obil'naya β-criptoxanthin was found to be the main carotenoid (18.4% of the total) [29], whereas the presence of zeaxanthin in seabuckthorn berries has also been reported by Yang [2].

During ripening the carotenoid fraction showed a gradual increase in all three cultivars (2.25-, 1.14- and 2.39-fold increase of total carotenoids in Askola, Hergo and Leikora, respectively). An approximately linear increase in total carotenoids (determined by spectrophotometric measurements) was previously observed by Gao and colleagues [5] in three Russian cultivars over 19 days of ripening (increases ranging from 1.6- to 6.5-fold). The pattern of variation from July till the end of August was similar in cultivars Askola and Leikora: zeaxanthin gradually and markedly accumulated (a 2.3-fold increase in Askola and a twofold increase in Leikora), and a similar trend was also observed for β-criptoxanthin (2.9- and 3.3-fold increase, respectively) and β-carotene (1.8- and 2.8-fold increase). Moreover, in Askola β-criptoxanthin and β-carotene, unlike zeaxanthin, went on accumulating even after the end of August. On the other hand, in Hergo zeaxanthin content showed only a slight increase from mid-July to the end of August (1.2-fold increase), whereas β-criptoxanthin and β-carotene did not show significant changes.

Seabuckthorn total carotenoid content was similar to that in other plant foods particularly rich in carotenoids (containing more than 100 mg/kg), such as tomatoes, carrot, kale and spinach [31]. In particular, high levels of zeaxanthin plus lutein have been reported in kale (147–395 mg/kg), spinach (44–159 mg/kg) and broccoli (18–20 mg/kg). Among carotenoids detected in the seabuckthorn berries analysed in our study, only β-carotene and β-criptoxanthin possess provitamin A activity. Concerning other possible physiological effects of seabuckthorn carotenoids, Yang and colleagues [8], in their study on the effect of dietary supplementation with seabuckthorn pulp oil on atopic dermatitis, suggested that the high content of carotenoids in the pulp oil could have contributed to the observed significant improvement of symptoms. Moreover, it has been reported that a diet rich in zeaxanthin and lutein increased their accumulation in the macula and that these carotenoids could protect against age-related macular degeneration, by preventing light-initiated oxidative damage to the retina and retinal pigment epithelium [32]. Furthermore, increasing frequency of intakes of foods rich in lutein and zeaxanthin has been associated with a moderate decrease in risk of cataract [33].

In a previous study carried out by Albrecht [34] on the cultivars Hergo and Leikora, aimed to determine the optimal harvest date, five traits were selected as having a major influence: fruit colour, total acidity, fruit weight, crop losses caused by mechanical harvesting and force needed to remove the berries from the branches. More recently, research on seabuckthorn berry quality has focused on chemical constituents related to the organoleptic properties and on bioactive compounds [2, 4, 5, 6, 12, 13, 16].

Our results confirmed that ripening was accompanied by a marked decrease in organic acids level and, in some cases, by an increase of the sugar:acid ratio, which could improve the taste attributes of the berry. On the other hand, when considering bioactive compounds a more complex picture appeared, the various classes of antioxidants showing quite different patterns of variation during ripening. According to previous observations on other genotypes and in quite different climatic conditions, ascorbic acid concentration markedly declined, and the highest levels were found in the early stages of ripening (in the environmental conditions of our experiment, in early or mid-July). As in other carotenogenic fruits, ripening was generally accompanied by enhanced carotenoid biosynthesis, which gave place to increasing carotenoid concentrations until full ripeness was achieved (in our case, at the end of August), despite the intense water uptake into the berries occurring at the same time. The genotype seemed to influence the extent of carotenoid accumulation, the carotenoid profile and, consequently, the proportion of vitamin A-active constituents of the whole fraction. Finally, although the pattern of variation of flavonols was less uniform among different genotypes, at each ripening stage the flavonol content was so high that the consumption of seabuckthorn products could significantly contribute to the dietary intakes of flavonols.

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