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Influence of origin, harvesting time and weather conditions on content of inositols and methylinositols in sea buckthorn (*Hippophaë rhamnoides*) berries

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ABSTRACT

Inositols and methylinositols play an important role in human physiology. Inositols and methylinositols in berries of three subspecies of sea buckthorn (*Hippophaë rhamnoides*) were analysed using gas chromatography combined with a flame ionisation detector and mass spectrometry. The wild Chinese berries (*H. rhamnoides* ssp. *sinensis*) contained higher levels of L-quebrachitol (1L-2-*O*-methyl-*chiro*-inositol) and methyl-*myo*-inositol (average 615 and 58 mg/100 ml juice, respectively) than the Finnish (*H. rhamnoides* ssp. *rhamnoides*, 276 and 11 mg/100 ml juice, respectively) and the Russian (*H. rhamnoides* ssp. *mongolica*, 228 and 16 mg/100 ml juice, respectively) berries ($P < 0.001$). The content of *myo*-inositol was higher in the Chinese and the Russian berries than in the Finnish berries (26 and 20 mg/100 ml juice vs. 8 mg/100 ml juice, $P < 0.001$). In the Chinese berries, the contents of methyl-*myo*-inositol and L-quebrachitol increased, whereas that of *myo*-inositol decreased from late September to late November. The content of the L-quebrachitol in the Chinese berries correlated negatively with the air temperature and the number of frost-free days, suggesting a possible role of the compound in the cold resistance of sea buckthorn.

1. Introduction

The positive effects of sea buckthorn (*Hippophaë rhamnoides*) berry and berry fractions on human health have been widely shown by both traditional use and modern research. Sea buckthorn berry has long been used in traditional Tibetan and Mongolian medicines for over 1000 years. It is included in the Chinese Pharmacopoeia. Results of clinical intervention studies as well as investigations using *in vitro* and animal models suggest that sea buckthorn berries and juice may improve lipid and sugar metabolism (Koyama, Taka, & Togashi, 2009; Nemes-Nagy et al., 2008), support cardiovascular health (Larmo, Alin, Salminen, Kallio, & Tahvonen, 2008; Liu et al., 1985), reduce oxidative stress (Eccleston et al., 2002; Gupta & Flora, 2006; Nemes-Nagy et al., 2008), combat the genotoxicities and cytotoxicities of chemicals and heavy metals (Nersesyan & Muradyan, 2004; Padmavathi et al., 2005; Xu et al., 2005), and inhibit the growth of tumour and cancer cells (Boivin, Blanchette, Barrette, Moghrabi, & Beliveau, 2007).

Active research has been carried out around the world to investigate the composition and bioactive components of sea buckthorn berries. The majority of the research has so far concentrated on components commonly found in fruits and berries such as sugars, acids, phenolic compounds, vitamin C, tocopherols, tocotrienols, fatty acids, carotenoids, and plant sterols (Kallio, Yang, & Peippo, 2002; Raffo, Paoletti, & Antonelli, 2004; Rosch, Krumbein, Mugge, & Kroh, 2004; Yang, Karlsson, Oksman, & Kallio, 2001; Yang, Linko, Adlercreuts, & Kallio, 2006). Compositional information limited to these components seems insufficient to interpret the exceptionally wide and significant physiological effects observed of sea buckthorn berries and juice. Further investigation searching for new biological active compounds is crucial for understanding the mechanisms behind the health benefits observed of sea buckthorn.

Inositols consist of nine cyclitol isomers, of which *myo*-, D-*chiro*-, L-*chiro*-, *scyllo*-, *muco*-, and *neo*-inositols have been found in plants (Loewus & Murthy, 2000). *Myo*- and *chiro*-inositols are essential components of phosphotidylinositols in cell membranes, which are precursors of second messengers for intracellular signal transduction. Inositol phosphoglycans containing *myo*- and *chiro*-inositols are believed to mediate insulin sensitivity and regulate carbohydrate metabolism *in vivo* (Larner, 2002).

Myo-inositol is converted to *chiro*-inositol *in vivo* (Larner, 2002). Proper dietary intake and metabolism of inositols especially *myo*- and *chiro*-inositols are crucial for a wide range of aspects of human physiology. For example, reduced levels of D-*chiro*-inositol was observed in patients with diabetes mellitus possibly resulting from reduced dietary intake of *myo*-inositol, impaired conversion of *myo*-inositol to *chiro*-inositol and/or increased urinary secretion of D-*chiro*-inositol (Larner, 2002; Ostlund et al., 1993).

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When administered i.v. to diabetic and insulin resistant rhesus monkeys, D-*chiro*-inositol increased the insulin sensitivity and the activation of glycogen synthase in muscles (Larner, 2002; Ortmeyer, Huang, Zhang, Hansen, & Larner, 1993; Ortmeyer, Bodkin, Hansen, & Larner, 1995). Oral administration of D-*chiro*-inositol increased insulin sensitivity in patients with the polycystic ovary syndrome, improved ovulatory function and decreased serum androgen concentrations, blood pressure, and plasma triglyceride level (Nestler, Jakubowicz, Reamer, Gunn, & Allan, 1999).

Depletion of *myo*-inositol in nerves has been suggested to be associated with the pathogenesis of diabetic neuropathy (Ziegler, 2008). Oral supplementation of *myo*-inositol showed chemopreventive potential against lung cancer and reduced both the systolic and diastolic blood pressures in smokers (Lam et al., 2006). Oral treatment with *myo*-inositol, D-*chiro*-inositol, or 3-*O*-methyl-D-*chiro*-inositol (pinitol) reduced hyperglycaemia and hypertriglyceridemia and prevented endothelial dysfunction in diabetic rats (Nascimento et al., 2006). L-Quebrachitol attenuated 6-hydroxydopamine-induced cytotoxicity in rat foetal mesencephalic cell cultures (Nobre Junior et al., 2006).

Recently, we have identified L-quebrachitol (1L-2-*O*-methyl-*chiro*-inositol) and *myo*-inositol and reported the presence of a *chiro*-inositol and a methyl-*myo*-inositol in Chinese sea buckthorn (*H. rhamnoides* ssp. *sinensis*) berry (Kallio et al., 2009). These components may play an important role in the physiological effects and sensory properties of sea buckthorn berries. The aim of the current research is to investigate the content of the inositols and methylinositols in berries of wild and cultivated sea buckthorn of three different subspecies, *H. rhamnoides* ssp. *sinensis*, ssp. *rhamnoides*, and ssp. *mongolica* harvested from Finland, China and Russia. Furthermore, the changes of the contents of these compounds during the harvesting period and the influence of the geographical and weather conditions at the growth sites were studied with the wild Chinese berries (*H. rhamnoides* ssp. *sisnensis*).

2. Materials and methods

2.1. Sea buckthorn berries

Wild berries of *H. rhamnoides* ssp. *sinensis* were picked from different natural growth sites in China during 1996–1998. In order to follow the changes during the harvesting period, wild berries of *H. rhamnoides* ssp. *sinensis* from two natural growth sites in China were collected at different harvesting dates during the period from August to November 1998. Table 1 presents the geographical and weather conditions (average values of the years 1989–1998 and values of each collecting year during 1996–1998) at the growth sites of the Chinese berries investigated in the present study.

Wild berries of *H. rhamnoides* ssp. *rhamnoides* were picked from the Baltic coast in south-western Finland in 1999. Cultivated berries of *H. rhamnoides* ssp. *rhamnoides* were harvested from the cultivation sites in Satakunta (south-western Finland) and Viikki (Helsinki, Finland) in 1999. Berries of seven commercial cultivars of *H. rhamnoides* ssp. *mongolica* were picked from Novosibirsk, Russia (Ruet, Luchezarnaya, Dar Katuni, Vitaminaya, and Maslichnaya) in 1997 and from Riihimäki (Oranzevaya and Tsuiskaya), Finland in 1999. The Finnish cultivars S3003, S3006, 74006003, 74006005 and 72004004 were selected by the Horticultural Research Institute, MTT Agrifood Research Finland (Piikkiö, Finland). S3003 and S3006 were two individuals selected from seedlings of X-ray irradiated seeds of wild Finnish berries (*H. rhamnoides* ssp. *rhamnoides*). 74006003, 74006005 and 72004004 were crossings between a wild Finnish male bush (*H. rhamnoides* ssp. *rhamnoides*) and a wild German female bush (*H. rhamnoides* ssp. *rhamnoides*). The berries cultivated in Viikki included one Chinese (*H. rhamnoides* ssp. *sinensis*) clone, two Finnish (*H. rhamnoides* ssp. *rhamnoides*) clones, one Danish (*H. rhamnoides* ssp. *rhamnoides*) clone, three crossings between Finnish and Danish clones, a crossing between Finnish and Chinese clones as well as two crossings between Finnish and Siberian clones (*H. rhamnoides* ssp. *mongolica*).

Table 2 presents a summary of the berry samples analysed in the current study. The berries were picked optimally ripe and loosely frozen immediately after picking. The berries were transported to Finland under freezing condition and kept at −20 °C until analysis within half year after the collection. Berries of each sample were pulled and analysed in quadruplicate from a 3–5 kg lot following a sample partitioning procedure.

2.2. Reagents

Reference compound L-quebrachitol (1L-2-*O*-methyl-*chiro*-inositol) was purchased from Alexis Corporation (Läufelfingen, Switzerland), *myo*-inositol from Fluka Chemie AG (Buchs, Switzerland). D-(+)-*chiro*-inositol and L-(−)-*chiro*-inositol from Sigma–Aldrich (Steinheim, Germany). Internal standard D-sorbitol was from Merck (Darmstadt, Germany). Formic acid was purchased from J.T. Baker (Deventer, Holland). Tri-Sil (trimethyl chlorosilane and hexamethyl disilazane in pyridine) was from Pierce Chemical Co (Rockford, IL).

2.3. Sample preparation

Thirty grams of sea buckthorn berries were thawed in a microwave oven (225 W) with 30% of power twice over 15 s and shaken out in between. The berries were crushed with a spoon and juice was pressed and filtered with cheesecloth. The filtrated juice sample was used for the isolation of the sugar fraction.

2.4. Isolation of sugar fraction and preparation of trimethylsilyl ethers

Sugar fraction was isolated from the juice and analysed according to the method applied earlier in this laboratory (Yang, 2009). One millilitre of filtrated juice sample was taken, followed by addition of 1 ml of water solution of sorbitol (2.0 g/100 ml) as the internal standard, 6 ml of 0.1 N NaOH solution, and 12 ml of Mili Q water. For isolation of sugars fraction, 350 μl of the dilution was applied on a preconditioned anion exchange Isolute SAX column (International Sorbent Technology, Hengoed, UK). The sugar fraction was eluted from the column with 2 ml of Milli Q water and diluted to a final volume of 3 ml. A sample of 1–2 ml was taken from the dilution and evaporated to dryness and left overnight in a desiccator. Trimethylsilyl (TMS) derivatives of sugars and sugar derivatives were prepared in a screw-cap glass vial sealed with a butyl Teflon septum. An aliquot of 200 μl of the reagent Tri-Sil was added to the vial; the vial was tightly closed and shaken vigorously on a Vortex (Vortex-Genie, Springfield, MA) for 5 min; thereafter, the vial was incubated at 60 °C for 30 min followed by storage at room temperature overnight before gas chromatographic analysis.

2.5. Identification of inositols and methylinositols

The TMS derivatives were analysed by gas chromatography–mass spectrometry (GC–MS) using a Shimadzu 17 A gas chromatograph equipped with a QP 5000 MSD detector (Shimadzu, Japan) and controlled by Class-5000 software. A Supelco Simplicity-1 fused silica capillary column (30 m, id 0.25 mm, film thickness 0.25 μm) (Bellefonte, PA) was used for the analysis. Helium was used as the carrier gas. Temperature programme of the column was as follows: hold at 90 °C for 2 min, increase from 90 °C to 275 °C at a rate of 4 °C/min, hold at 275 °C for 10 min. The