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Tissue biochemical diversity of 20 gooseberry cultivars and the effect of ethylene supplementation on postharvest life

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ABSTRACT

The European gooseberry (*Ribes uva-crispa*) is still an understudied crop with limited data available on its biochemical profile and postharvest life. A variety of polyphenols were detected in the skin and flesh of 20 gooseberry cvs, representing mainly flavonol glycosides, anthocyanins and flavan-3-ols. In contrast, gooseberry seeds were for the first time characterised by the presence of considerable amounts of hydroxycinnamic acid glycosides tentatively identified by UPLC-QToF/MS. All cvs examined represented a good source of vitamin C while being low in sugar. Furthermore, the postharvest stability of bioactives was explored by supplementation of exogenous ethylene in air at 5 °C. Results suggest a low sensitivity of gooseberries to ethylene. The overall quality of gooseberries remained stable over two weeks, showing potential for extended bioactive life.

1. Introduction

Gooseberries are deciduous shrubs being members of the *Grossulariaceae* family and genus *Ribes* like blackcurrants, redcurrants, whitecurrants and jostaberries (Bordonaba and Terry, 2011). The genus is comprised of more than 150 diverse species with currants and gooseberries being the most popular (Barney and Hummer, 2005). *Ribes uva-crispa* L. (synonym *Ribes grossularia* L.) is a European species and the most prevalent species among the gooseberries found across the world. It is native to United Kingdom, Caucasus Mountains and North West Africa (Barney and Hummer, 2005). The size of gooseberries varies as does their skin colour ranging from green to pink, red, purple, white, and yellow (Hummer and Dale, 2010). The commercial value of gooseberries is limited at present, mainly due to low demand and high cost of production especially during harvesting (Barney and Hummer, 2005; Dale, 2000). Other influencing factors include prevalence of crop diseases such as powdery mildew (Barney and Hummer, 2005) and lack of high yielding cvs (Pluta et al., 2010).

In recent years there is a rising trend in domestic cultivation of *Ribes* berries both in Europe and other regions (Barney and Hummer, 2005; Mitchell et al., 2011). Reasons for the increasing interest include small agricultural requirements, resistance to cold winters and the development of improved cvs with better disease resistance, colour, flavour and yield. Quality of gooseberries is primarily based on its visual, textural, organoleptic and nutritional characteristics (Terry et al., 2009). The later attribute has attracted considerable interest over the years, as some bioactive components in berries have been associated with potential health-promoting properties (Bordonaba and Terry, 2011; Folmer et al., 2014; Wang and Stoner, 2008).

Despite the amount of information on the qualitative and quantitative content of bioactives in berries, the nutritional quality of gooseberries has not yet been sufficiently explored due to limited commercial interest. Only a few gooseberry cvs have been studied thus far in which, polyphenols have been extracted from the fruit as a whole (Jordheim et al., 2007; Määttä-Riihinen et al., 2004; Pantelidis et al., 2007; Wu et al., 2004). There is limited information about the spatial contribution across tissues in the final polyphenolic content of gooseberries. The main phenolic compounds reported in gooseberries include anthocyanins (Jordheim et al., 2007; Määttä-Riihinen et al., 2004; Pantelidis et al., 2007; Wu et al., 2004), flavonol glycosides and proanthocyanins

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(Chiang et al., 2013; Häkkinen et al., 1999a; Mikulic-Petkovsek et al., 2012a; Russell et al., 2009).

The limited commercial value of gooseberries is also depicted in the scarce data available on the postharvest life of gooseberries and the stability of their presumed bioactive components during storage. At present, only a few reports exist on the physical, physiological and biochemical changes occurring in gooseberries during different storage conditions (Harb and Streif, 2004; Kampuse et al., 2015; Muizniece-Brasava et al., 2015). In addition, a better understanding is needed on the role of ethylene in the postharvest life of gooseberries. Gooseberries are classified as non-climacteric fruits, although they are able to produce ethylene in low amounts (0.035–0.35 ng kg^{-1} s^{-1} at 20 °C) (Cantwell, 2002; Thompson, 2002). The role of ethylene on *Ribes* berries, however, has not been thoroughly investigated with reports often contradictory regarding their sensitivity (Cantwell, 2002; McKay and Van Eck, 2006).

The objective of this study was thus two-fold: to explore the biochemical profile of different tissues of a wide selection of gooseberry varieties grown in the UK, and elucidate postharvest changes in biochemistry and quality characteristics for two gooseberry varieties held for 15 days at low temperature with or without application of exogenous ethylene.

2. Material and methods

2.1. Chemicals

All HPLC and LC–MS grade solvents were obtained from Fisher Scientific (Loughborough UK). (+)-catechin, (−)-epicatechin, procyanidin B1, procyanidin B2, neochlorogenic acid, caffeic acid, sinapic acid, *p*-coumaric acid, quercetin-rutinoside, quercetin-glucoside, isorhamnetin-glucoside, isorhamnetin-rutinoside were purchased from Sigma–Aldrich (Dorset, UK). Cyanidin-3-glucoside and cyanidin-3-rutinoside, were purchased from Extrasynthese (Genay Cedex, France). Metaphosphoric acid (Bioxtra ≥ 33.5%), L-ascorbic acid and D-fructose were obtained from Sigma–Aldrich (Dorset, UK). D-glucose and sucrose, were purchased from Fisher Scientific (Loughborough UK).

2.2. Plant material and sample preparation

Gooseberry fruits from 20 cvs of *R. uva-crispa* were obtained at optimum maturity, from The National Fruit Collection (Brogdale, Kent, UK) on the 13th of July 2012 for biochemical analysis (Fig. 1). Based on the biochemical results obtained, two cvs 'Careless (Kent)' and 'Scotch Red Rough' were selected and harvested again the following year (12th of July 2013) for the purposes of the postharvest trial. Approximately 100–200 berries were harvested from two plants per cv at optimum maturity stage and transported to Cranfield University in cool boxes within 2 h from collection and immediately snap-frozen in liquid nitrogen. The samples were divided into triplicates and stored at −80 °C before analysis. Each sample was further divided into two subsamples. Half of the material (approximately 100 g) was freeze-dried, the seeds were manually removed and the berries were ground into a fine powder for the extraction of phenolics and sugars. The second subsample was kept fresh frozen and powdered in a mortar grinder (RM 200, Retsch Ltd., Derbyshire, UK) with liquid N_2 and used for the extraction of ascorbic acid to avoid potential degradation of ascorbic acid during the freeze-drying process.

2.3. Postharvest trial

Samples from the two selected gooseberry cvs ('Scotch Red Rough' and 'Careless (Kent)') were transferred inside polystyrene boxes with ice-packs, from The National Fruit Collection to Cranfield within 3 h from harvest. Upon arrival at Cranfield, gooseberries from each cv were split into two batches. The four batches of gooseberries were placed in plastic stackable crates inside water sealed, air-tight polypropylene chambers (88 cm × 59 cm × 59 cm) fitted with two 8 × 8 cm electric fans (Nidec beta SL, RS Components Ltd., Northants, UK) to circulate the ethylene gas during treatment. Two boxes per cv were injected with 11.69 μg L^{-1} of standard ethylene gas (100% ethylene; SIP analytical) and the other two boxes were used as control samples (untreated). Temperature of the storage room was set at 5 °C and the treatment time was 24 h. The concentration of the ethylene gas in the chambers was confirmed after 30 min of injection and after 24 h of storage by withdrawing air from the chambers (including controls), using a tapped 20 mL plastic syringe. The headspace of the sampled air was injected into a gas chromatograph (GC—Model 8340, DP800 integrator, Carlos Erba Instruments, Herts, UK, analytical column, Porapak) fitted with flame ionisation detector (FID) and ethylene gas present quantified.

After the treatment was completed (24 h), every batch was split into three replicates and stored in ventilated propylene (15 cm × 22 cm × 8 cm) containers. The containers had an inlet and outlet that provided ventilation to the fruits by way of pumping air into the boxes at a flow rate of about 3.33 mL s^{-1} from a flowmeter controlled unit attached to a ICA 6000 (International Controlled Atmosphere Ltd., Kent, UK). The RH inside the boxes was maintained at 80 ± 10% by placing a beaker of water inside the containers. The RH and temperature in the boxes was continuously monitored using Tinytag Ultra 2 TGU-4500 data loggers (−95% RH, −25 to 85 °C, Gemini Data Loggers Ltd., West Sussex, UK).

2.3.1. Sampling during storage period

Upon arrival (Day 0), five gooseberries were randomly selected from each cv in triplicate. After the treatment (Day 1), five gooseberries were sampled randomly from each box (3 boxes per treatment). Sampling was thereafter repeated at regular intervals (Day 4, Day 7, Day 11, Day 13, Day 15). All samples were subjected to colour and ethylene production measurements before being snap-frozen in liquid nitrogen. The snap-frozen berries were stored at −80 °C until analysis. Prior to extraction the plant material was freeze-dried and powdered as before and analysed for individual soluble sugars and phenolics. Water content of gooseberries was calculated by recording the weight of all samples before and after freeze-drying.

2.4. HPLC-ELSD analysis of non-structural carbohydrates

Extraction of non-structural carbohydrates was performed according to a previous method with slight modifications (Terry et al., 2007). Prior to analysis, the sugars extracts were diluted (1:9, v/v) with HPLC grade water and injected into an Agilent 1200 HPLC fitted with a prevail carbohydrate ES 5 μm size of 250 nm × 4.6 mm diameter and a guard column of the same type. The mobile phase consisted of solvent A (water) and solvent B (acetonitrile) and the elution gradient was as follows: 0–15 min, 80–50% B, 15–20 min, 50–20% B, 20–25 min, 80% B. The eluted compounds were detected by evaporative light scattering detector (ELSD) and quantification was based on external calibration curves of commercial standards.

2.5. Phenolic compounds

2.5.1. Extraction of phenolic compounds

Extraction of phenolic compounds from berries and seeds was performed according to a previous method with slight modifications (Giné Bordonaba and Terry, 2008). Freeze–dried berry powder (150 mg) and freeze–dried seed powder (50 mg) were