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HYDROXYBENZOIC AND HYDROXYCINNAMIC ACID DERIVATIVES IN SOFT FRUITS

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Key Word Index—Rosaceae; Saxifragaceae; blueberries; fruits; hydroxybenzoic acids; hydroxycinnamic acids; esters; glucosides.

Abstract—Quinic acid and glucose esters of hydroxycinnamic acids and glucosides of hydroxycinnamic and hydroxybenzoic acids have been determined by capillary GC and HPLC in soft fruits. The results confirm the wide distribution of these compounds in fruit tissues.

INTRODUCTION

Quinic acid and glucose esters of hydroxycinnamic acids are widely distributed in fruit [1–3] and vegetable [4–6] species. There is, however, little information on the corresponding esters of hydroxybenzoic acids, except gallic acid, and on the glycosides of phenolic acids. The 4-β-D-glucosides of the three hydroxycinnamic acids occur in tomatoes [7, 8]. Caffeic acid 3-β-D-glucoside was reported in leaves of *Populus nigra* [9] and in berries of wild tuberous potato species [10], caffeic acid 4-β-D-glucoside in cranberries [11], protocatechuic acid 4-β-D-glucoside in cockroaches (*Periplaneta americana* and *Blatta orientalis*) [12], protocatechuic acid 3-β-D-glucoside in *Pyrus* species [13], and syringic acid 4-β-D-glucoside in *Anodendron affine* [14]. The β-D-glucosides of *p*-coumaric, ferulic, *p*-hydroxybenzoic, protocatechuic and vanillic acids have previously been found in needles of conifers, while *p*-hydroxybenzoic acid β-D-glucoside has been found to occur widely in the Apiaceae [15].

To obtain more information on the occurrence of hydroxycinnamic and hydroxybenzoic acid derivatives in soft fruits, we have here analysed these tissues by GC and HPLC.

RESULTS AND DISCUSSION

Table 1 lists the quinic acid and glucose esters of hydroxycinnamic acids and gallic acid found and Table 2 the glucosides of phenolic acids. The new IUPAC nomenclature for the quinates (e.g. chlorogenic is the 5′-ester) is used. All the fruits analysed were fully ripe and therefore in a comparable state of maturity.

The results of the analyses by the two methods employed for glucosides (see Experimental) were in close agreement with each other. Hydroxycinnamoylglucoses could not be analysed by GC because *trans*-esterification occurs during analysis. For determination of *p*-hydroxybenzoic acid β-D-glucoside by GC the compounds have to be separated from interfering substances by anion exchange. During evaporation of eluates containing formic acid the glucosides can undergo partial hydrolysis. Therefore, HPLC is preferable to GC in this case.

Our investigations show a wide distribution of phenolic acid glucosides. In raspberries and blackberries the β-D-glucosides of *p*-coumaric, *p*-hydroxybenzoic and protocatechuic acids occur regularly. In raspberries the content of *p*-hydroxybenzoic acid β-D-glucoside is distinctly higher than in other rosaceous fruits. Fruits of the Saxifragaceae—currants, gooseberries and Josta—contain *ca* 2–10 ppm of all three phenolic acid glucosides. In contrast to the other phenolic compounds the contents of *p*-hydroxybenzoic acid β-D-glucoside and *p*-coumaric acid β-D-glucoside are somewhat higher in redcurrants than in blackcurrants. Higher amounts of glucosides were found in the high-bush blueberry, the only species containing merely traces of glucose esters.

Hydroxycinnamoylglucoses were found in all berries investigated (Table 2), but in varying amounts. Galloylglucose (β-D-glucogallin) occurred in blackberries and blackcurrants (2–7 ppm) and in traces in raspberries and Josta.

Among the quinates (Table 1), caffeic acid esters are predominant. In blackcurrants, Josta and blackberries 3′-caffeoylquinic acid is the main component. In blueberries 5′-caffeoylquinic acid is the major constituent. Further, small amounts of 5′-*p*-coumaroylquinic acid and 5′-feruloylquinic acid were detected. Traces of 5′-galloylquinic acid (theogallin) were found in strawberries, raspberries, redcurrants and blackcurrants.

EXPERIMENTAL

Extraction and purification. Fresh fruit (100 g) was homogenized with 80% MeOH, made up to *ca* 600 ml with more 80% MeOH, and extracted for 30 min with stirring at room temp. The extraction was repeated, the extracts combined and evaporated under red. pres., to an aq. residue (100 ml), which was purified on

Table 1. Contents (ppm fr. wt) of hydroxycinnamoyl and galloylquinic acids (by GC) and glucose esters (by HPLC) in soft fruits

Fruit cultivar*	5′-CafQ	4′CafQ	3′-CafQ	5′-pCoumQ	4′-pCoumQ	3′-pCoumQ	5′-FerQ	4′-FerQ	3′-FerQ	CafG	pCoumG	FerG	GalG	GalQ
Strawberries														
Tago	–	–	–	–	–	–	–	–	–	+	14	+	–	1
Litessa	–	–	–	–	–	–	–	–	–	2	27	2	–	+
Raspberries														
Malling Exploit	1	–	–	2	–	–	+	–	–	3	6	4	+	+
Glen Cova	+	–	–	1	–	–	+	–	–	7	14	7	+	+
Blackberries														
Thornless														
Evergreen	3	1	43	–	–	4	+	–	2	6	5	5	2	–
Black Thornfree	3	1	41	–	–	2	–	–	4	6	11	6	3	–
Theodor Reimers	+	1	52	–	–	5	–	–	3	3	4	2	3	–
Redcurrants														
Fay's Prolific	1	–	+	–	–	+	2	–	–	5	2	+	–	+
Red Lake	1	–	+	–	–	1	2	–	–	3	+	+	–	+
Heinemanns														
Spätlese	1	–	–	–	–	–	1	–	–	2	+	+	–	+
Blackcurrants														
Ni 70	2	5	38	–	1	13	1	3	+	21	10	13	5	1
Ni 76	1	3	48	–	1	18	1	2	1	30	14	15	7	1
Silmu	2	3	43	–	2	21	2	2	1	19	10	11	4	+
Gooseberries														
Yellow Triumph	–	–	3	–	–	1	–	–	1	5	7	+	–	–
Mauks Early Red	–	–	4	–	–	+	–	–	1	13	7	6	–	–
Josta	+	8	26	–	–	14	+	2	2	35	15	32	+	–
Blueberries (high-bush)														
Bluecrop	1851	2	7	2	–	–	7	–	–	+	+	+	–	–
Heerma	2075	5	5	5	–	–	8	–	–	+	+	+	–	–

+, Trace; –, not detectable; Josta, a hybrid of blackcurrant and gooseberry. CafQ, Caffeoylquinic acid; pCoumQ, *p*-coumaroylquinic acid; FerQ, feruloylquinic acid; GalQ, 5′-galloylquinic acid; CafG, 1-*O*-caffeoyl-β-D-glucopyranose; pCoumG, 1-*O*-*p*-coumaroyl-β-D-glucopyranose; FerG, 1-*O*-feruloyl-β-D-glucopyranose; GalG, 1-*O*-galloyl-β-D-glucopyranose.

*Several other cultivars were analysed with similar results: strawberries, Optima, Demetra, Gourmella; raspberries, Jochen Roem, Lloyd George, Delight, Veten; blackberries, Blacky; redcurrants, Monster, Bad Gasteiner; blackcurrants, Ben Lomond, Green's Black.

Table 2. Contents (ppm ft. wt) of glucosides of hydroxycinnamic acids and hydroxybenzoic acids in soft fruits by GC and HPLC

Fruit variety	CafGluc HPLC	CafGluc GC	pCoumGluc HPLC	pCoumGluc GC	FerGluc HPLC	FerGluc GC	pHBGluc HPLC	pHBGluc GC	ProGluc HPLC	ProGluc GC	GalGluc HPLC	GalGluc GC
Strawberries												
Tago	–	–	+	+	–	–	6	7	–	–	–	–
Litessa	–	–	+	+	–	–	6	3	–	–	–	–
Raspberries												
Malling Exploit	–	–	4	5	+	–	44	41	+	+	–	+
Glen Cova	–	–	9	10	2	2	59	56	+	+	–	+
Malling Promise	–	–	6	6	–	–	34	32	+	2	–	+
Blackberries												
Thornless Evergreen	–	–	2	2	–	–	8	9	5	4	+	2
Black Thornfree	–	–	4	5	–	–	21	18	6	3	–	+
Theodor Reimers	–	–	2	2	–	–	5	4	2	2	–	–
Redcurrants												
Fay's Prolific	2	2	13	16	+	–	13	10	+	+	–	–
Red Lake	2	2	10	14	–	–	9	7	2	2	–	–
Heinemanns Spätlese	+	+	5	5	–	–	9	7	+	+	–	–
Blackcurrants												
Ni 70	2	3	3	4	3	4	5	4	+	+	+	+
Ni 76	2	+	7	10	3	4	4	4	2	2	3	2
Silmu	2	+	5	5	2	2	.13	10	–	+	–	+
Gooseberries												
Yellow Triumph	2	+	4	6	2	2	9	9	2	2	+	2
Mauks Early Red	2	2	6	8	4	7	14	14	7	6	+	2
Josta	2	3	8	11	4	6	10	9	4	4	+	3
Blueberries (high-bush)												
Bluecrop	3	4	4	3	5	10	6	5	4	6	7	9
Heerma	3	3	15	15	6	8	5	4	4	3	3	2

+, Trace; –, not detectable. CafGluc, Caffeic acid 4-β-D-glucoside; pCoumGluc, *p*-coumaric acid β-D-glucoside; FerGluc, ferulic acid β-D-glucoside; pHBGluc, *p*-hydroxybenzoic acid β-D-glucoside; ProGluc, protocatechuic acid 4-β-D-glucoside; GalGluc, gallic acid 4-β-D-glucoside.

polyamide (250 × 35 mm i.d.; MN-Polyamide-SC-6, 0.05–0.16 mm, Macherey–Nagel, Düren, W. Germany). After sample application the column was eluted with 500 ml H_2O. This fraction contained *p*-hydroxybenzoic acid glucoside. Then 800 ml MeOH were used to elute glucose esters and glucosides of hydroxycinnamic and hydroxybenzoic acids followed by 1000 ml MeOH–HCOOH (995:5) to elute hydroxycinnamoylquinic acids and galloylquinic acid.

For GC separation of *p*-hydroxybenzoic acid β-D-glucoside the aq. extract was applied to an anion exchange column (Dowex 1 × 2, 100–200 mesh, counter ion: $HCOO^-$). The system was flushed with 100 ml 50% MeOH, 25 ml Me_2CO–MeOH–H_2O (1:1:1), and 10 ml 90% Me_2CO. The glucoside was eluted with 150 ml Me_2CO–HCOOH–H_2O (18:1:1).

Analyses. Methods and equipment for GC and HPLC of quinates [16], HPLC of glucose esters and glucosides of phenolic acids [8] and HPLC of *p*-hydroxybenzoic acid β-D-glucoside [17] have been described elsewhere. Syntheses of protocatechuic acid 4-β-D-glucoside, gallic acid 4-β-D-glucoside, and hydroxycinnamic acid glucosides and confirmation of their constitution by chemical and spectroscopic methods will be described elsewhere. Hydroxycinnamoylquinic acids [2], hydroxycinnamoylglucoses [3] and galloylquinic acid from green tea were at our disposal as authentic substances. We received *p*-coumaroylglucose from Birkofer (University of Düsseldorf, W. Germany) and galloylglucose from Gross (University of Ulm, W. Germany) with many thanks.

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