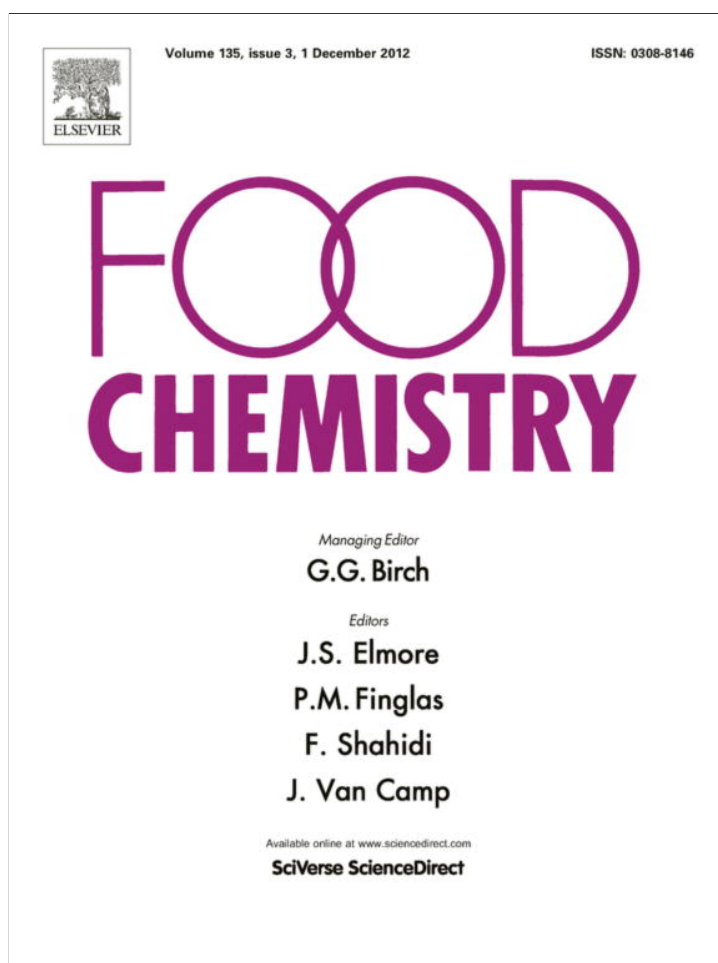


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## Berry components inhibit $\alpha$ -glucosidase *in vitro*: Synergies between acarbose and polyphenols from black currant and rowanberry

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## ABSTRACT

Polyphenol-rich extracts from certain berries inhibited  $\alpha$ -glucosidase activity *in vitro*. The two most effective berry extracts, from black currant and rowanberry, inhibited  $\alpha$ -glucosidase with IC<sub>50</sub> values respectively of 20 and 30  $\mu$ g GAE/ml and were as effective as the pharmaceutical inhibitor, acarbose. These berry extracts differed greatly in their polyphenol composition: black currant was dominated by anthocyanins (~70% of total) whereas rowanberry was enriched in chlorogenic acids (65% total) and had low levels of anthocyanins.

Both black currant and rowanberry extracts potentiated the inhibition caused by acarbose and could replace the inhibition lost by reducing the acarbose dose. However, no additive effects were noted when black currant and rowanberry extracts were added in combination. The mechanisms underlying the synergy between acarbose and the berry polyphenols and the lack of synergy between the berry components are discussed. These extracts exhibited the potential to replace acarbose (or reduce the dose required) in its current clinical use in improving post-prandial glycaemic control in type 2 diabetics. As a result, these polyphenols may offer a dietary means for type 2 diabetics to exercise glycaemic control.

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### 1. Introduction

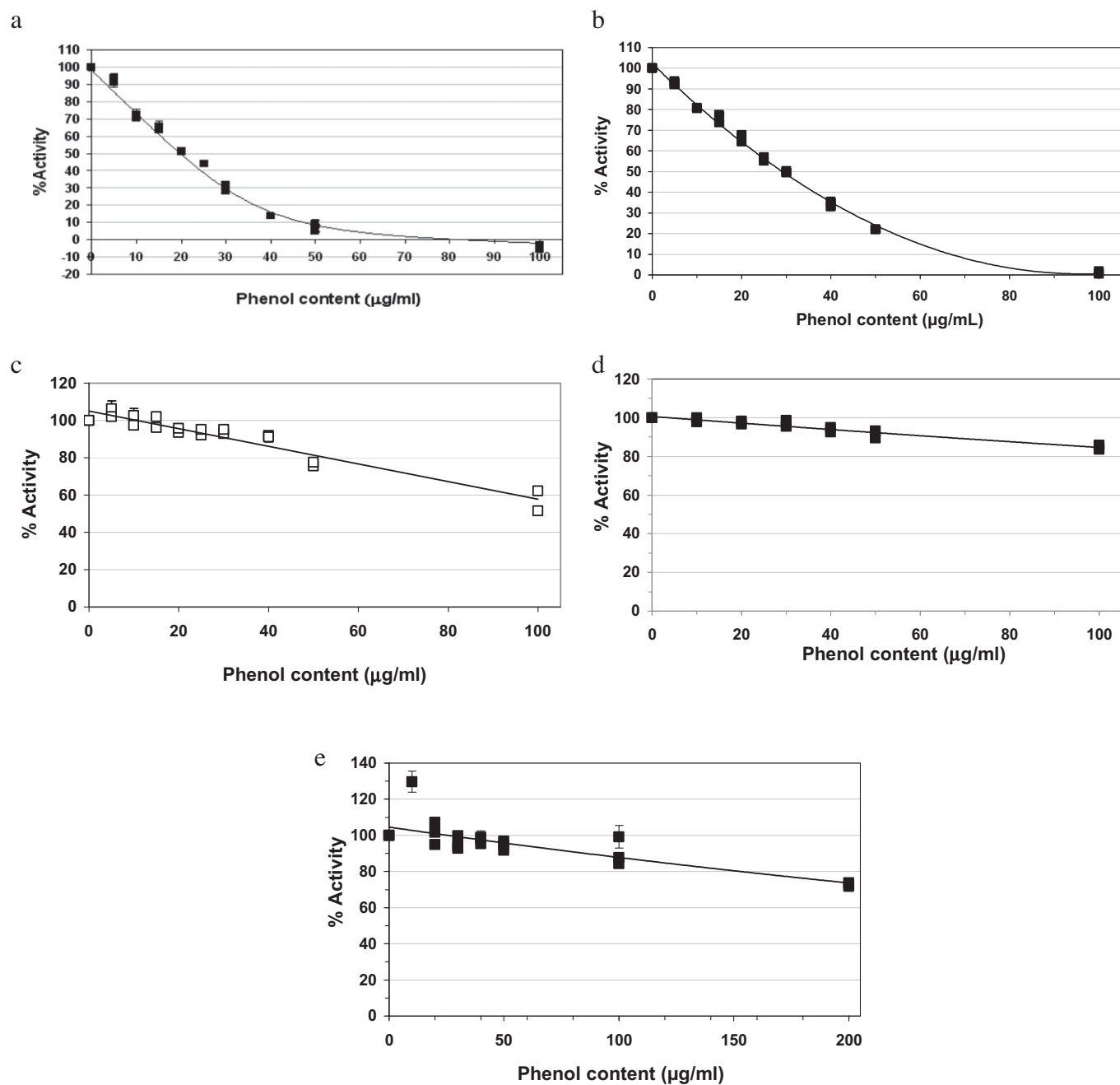
The World Health Organization (WHO) estimated that there are 346 million people worldwide who suffer from diabetes but this figure will double by the year 2030. Diabetes is a group of metabolic diseases but the predicted increase is mainly in type 2 diabetes, which has a number of lifestyle-related risk factors including smoking, obesity, poor diet and physical inactivity (Anon, 2011). Diets rich in fruit and vegetables (FAV) have been associated with reduced incidence of type 2 diabetes (Carter, Gray, Troughton, Khunti, & Davies, 2010) and some researchers have suggested that diet may ameliorate the poor glycaemic control associated with type 2 diabetes (e.g. Barnard et al., 2006). Indeed the level of diabetes is such that the associated European healthcare costs in 2010 were estimated to be \$196 billion, 10% of the total European health expenditure, with a predicted rise to almost \$235 Billion in 2030 (Zhang et al., 2010).

The effectiveness of FAV-rich diets may reflect overall reductions in sugar and fat intake but may also be due to increased intake of non-nutritive phytochemical components from FAV which could directly and beneficially influence health. Polyphenols are a major phytochemical component of fruits and are particularly

enriched in berries (Kahkonen, Hopia, and Heinonen, 2001). The theory is that the health benefits associated with a diet rich in FAV may be derived, in part, from the intake of natural antioxidants (Halliwell, 1996), such as polyphenols, has gained popularity. Polyphenols are proposed to protect against the damage caused by free radicals to DNA, membranes and cellular components, which are involved in disease progression. However, this theory has been challenged by the growing evidence that dietary polyphenols have substantially different bioavailabilities (Manach, Williamson, Morand, Scalbert, & Remesy, 2005). For example, anthocyanins, which are abundant in berries (Kahkonen et al., 2001), have low serum bioavailability and it has been argued that systemic bioactivities ascribed to anthocyanins may be mediated by their degradation products (Kay, Kroon, & Cassidy, 2009). Other major berry components, such as the tannins [ellagitannins and proanthocyanidins] also have poor serum bioavailability (Manach et al., 2005). As a result, a large proportion of potentially protective berry polyphenols are unable to enter the circulation and influence cellular interactions. In fact, most polyphenols from berries remain in the gastrointestinal tract (GIT) and pass through to the large intestine where they are subject to biotransformation by colonic microbiota (Williamson & Clifford, 2010). Therefore, one could propose that the health benefits derived from a diet rich in polyphenol antioxidants may be partly delivered through effects carried out within the GIT. In particular, evidence that polyphenols may modulate

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**Fig. 1.** Effect of berry extracts on  $\alpha$ -glucosidase activity. (a) Effect of black currant extracts on  $\alpha$ -glucosidase activity; (b) effect of rowanberry extracts on  $\alpha$ -glucosidase activity; (c) effect of rowanberry proanthocyanidin-rich extracts on  $\alpha$ -glucosidase activity; (d) effect of cloudberry extracts on  $\alpha$ -glucosidase activity; (e) effect of raspberry extracts on  $\alpha$ -glucosidase activity. The scatter plots contain data from three separate experiments. Values are % control activity  $\pm$  standard error. The trend lines were fitted by the Excel software.

nutrient availability through the inhibition of digestive enzymes involved in lipid and starch breakdown, which could influence obesity (McDougall, Kulkarni, & Stewart, 2009) and blood glucose control (McDougall, Kulkarni, & Stewart, 2008) respectively, has accrued. Polyphenol-rich extracts from berries inhibited the two main enzymes involved in starch digestion,  $\alpha$ -amylase and  $\alpha$ -glucosidase, *in vitro* and at levels achievable *in vivo* (McDougall et al., 2005). Indeed, both these enzymes are targeted by proprietary drugs, e.g. acarbose (trademarks Glucobay and Precose), which are prescribed to control blood glucose levels in type 2 diabetics after starch-containing meals (Rosenstock et al., 1998).

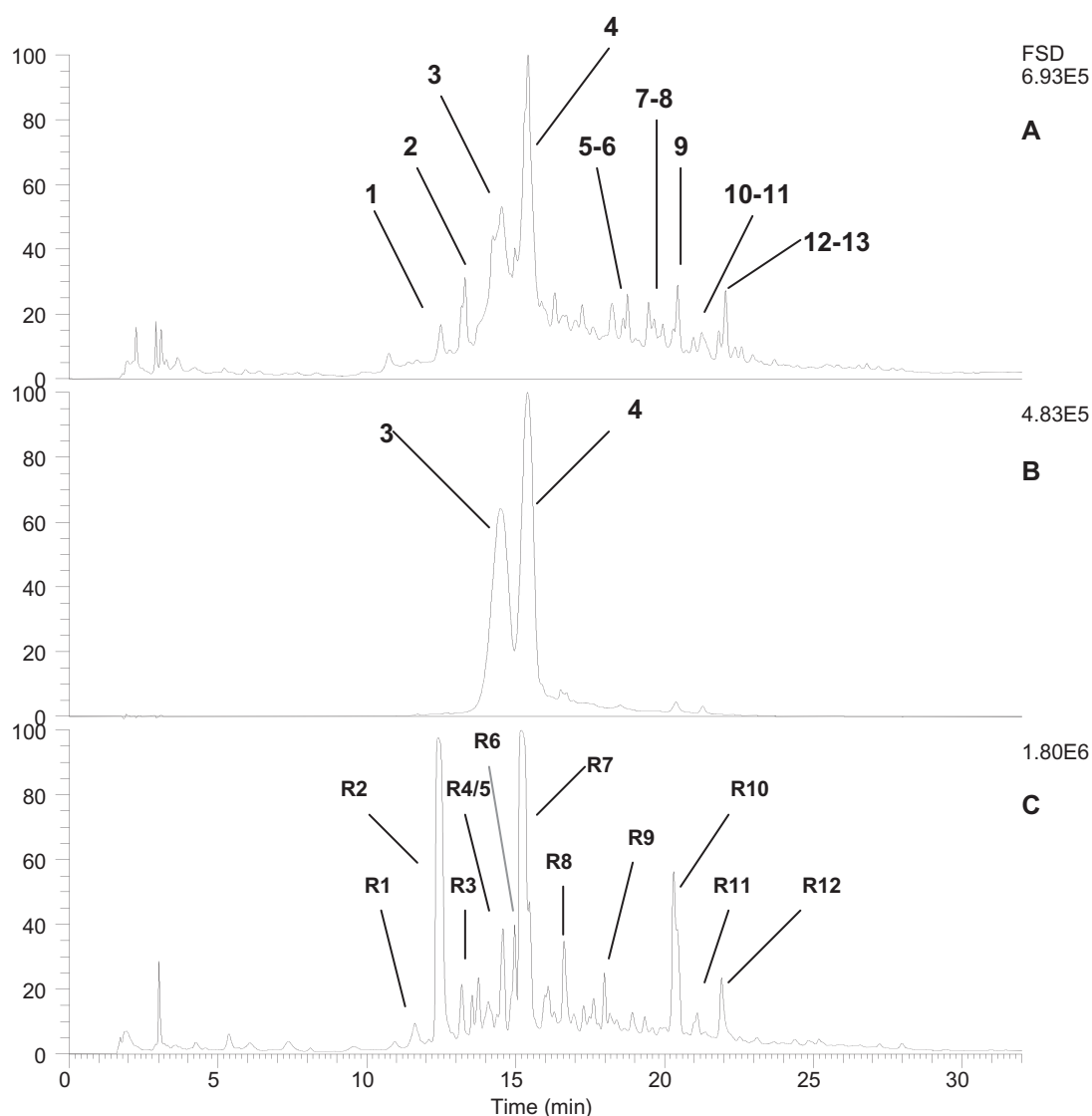
In recent work, we confirmed that polyphenol-rich extracts from berries could potentially inhibit  $\alpha$ -amylase *in vitro* (Grussu, Stewart, & McDougall, 2011) and identified candidate components

responsible for the inhibition. In addition, we demonstrated that berry polyphenols could act in concert with the therapeutically-used  $\alpha$ -amylase inhibitor, acarbose, to reduce the dose required for effective glycaemic control. In this study, we examine polyphenol-rich berry extracts for inhibition of  $\alpha$ -glucosidase *in vitro* and examine their interaction with acarbose.

## 2. Materials and methods

### 2.1. Plant material and extraction

Fruit were mainly obtained in the summer of 2008. Black currants (*Ribes nigrum* L. variety 8982-6) were obtained from Braden-



**Fig. 2.** Fig. 2 LC-UV traces of black currant and rowanberry extracts. (A) Trace at 280 nm of the black currant extract. Each injection contained 20  $\mu$ g GAE. The figure in the top left hand corner is the full scale deflection of the detector; (B) trace at 520 nm of the black currant extract; (C) trace at 280 nm of the rowan berry extract. All peak annotations refer to Table 1.

ham Hall, Norfolk, UK. Cloudberrries (*Rubus chamaemorus*) and rowanberries (*Sorbus aucuparia*, variety Sahharnaja) were obtained from Dr. Harri Kokko, University of Kuopio, Finland. Raspberries (*Rubus idaeus*, variety Glen Ample) were obtained from local farmers. All fruit were picked at full ripeness and frozen then transported frozen to JHI.

Fruits were extracted and polyphenol-rich extracts, devoid of minerals, sugars and vitamin C, were obtained by solid phase extraction (SPE) as outlined previously (Grussu et al., 2011). The SPE extracts were evaporated to dryness in a SpeedVac (Thermo Scientific, Waltham, MA). The proanthocyanidin-rich fraction was prepared from the rowanberry extract by sorption to Sephadex LH-20 as described previously (Grussu et al., 2011).

## 2.2. Total phenol assays

Phenol content was measured using a modified Folin-Ciocalteu method (see McDougall et al., 2005), estimated from a standard curve of gallic acid and expressed as gallic acid equivalents

(GAE). Samples were dried in aliquots to constant phenol content using a SpeedVac.

## 2.3. $\alpha$ -Glucosidase assay

The  $\alpha$ -glucosidase assay was described previously (Whitson et al., 2010) and used to measure inhibition of  $\alpha$ -glucosidase by berry extracts, pure compounds and pharmaceuticals. The buffer for this assay was 100 mM HEPES (pH 6.8) and the substrate was 2 mM *p*-nitrophenyl  $\alpha$ -D-glucopyranoside. The enzyme source was rat intestinal acetone powder (Sigma–Aldrich) dissolved in ultra pure water (UPW) at 10 mg/ml then centrifuged at 16,000 rpm for 5 min, and the supernatant used. Inhibitors were added in a fixed total volume to obtain the concentration ranges required for individual experiments. Controls lacking inhibitors were run and defined the control activity in each experiment. Each treatment was accompanied by a treatment blank containing all components apart from the enzyme to account for the possible absorbance of the berry extracts/inhibitors. This assay was time and substrate-sensitive therefore all components were added in a specific order;

**Table 1**  
Putative identification of peaks in black currant extracts.

Peak No.	Retention time	PDA maxima	<i>m/z</i>	MS <sup>2</sup>	Putative identity
1	12.45	325	353	191	Chlorogenic acid
2	13.29	315	341	179	Caffeoyl glucoside
3	14.55	525, 280	+465, 303 +611, 303	303 465, <b>303</b>	Delphinidin glucoside Delphinidin rutinoside
4	15.48	520, 280	<b>449</b> , 287 <b>595</b> , 287	287 449, <b>287</b>	Cyanidin glucoside Cyanidin rutinoside
5	18.27	355	<b>625</b> , 317	317	Myricetin rutinoside
6	18.76	355	<b>479</b> , 317	317	Myricetin glucoside
7	19.46	355	<b>521</b> , 317	479, <b>317</b>	Myricetin acetyl hexose
8	19.67	355	<b>609</b> , 301	449, <b>301</b>	Quercetin Rutinoside
9	20.44	355	<b>463</b> , 301	301	Quercetin Hexoside
10	20.97	355	<b>593</b> , 285	447, <b>285</b>	Kaempferol rutinoside
11	21.30	355	<b>505</b> , 301 <b>623</b> , 315	301 315	Quercetin acetyl hexose Isorhamnetin* rutinoside
12	21.82	355	<b>447</b> , 285	285	Kaempferol glucoside
13	22.05	315	565	519, <b>357</b>	Hydroxycinnamate derivative

\* – Other kaempferol or isorhamnetin derivatives may be present but at very low levels.+ = Positive mode. Figures in bold denote the dominant signal.

**Table 2**  
Putative identification of peaks in rowanberry extracts.

Peak No.	Retention time	PDA maxima	<i>m/z</i>	MS <sup>2</sup>	Putative identity
R1	11.61	330	353	191, 179	Caffeoyl quinic acid
R2	12.40	325	353	191, 179	5-Caffeoyl quinic acid
R3	13.21	310	341	179	Caffeoyl glucose
R4	13.75	320	707	<b>515</b> , 463, 323	Caffeoyl quinic acid derivative
R5	14.57	310	337	191, <b>163</b>	Coumaroyl quinic acid
R6	14.97	515, 280	<b>+419</b> , 287	+287	Cyanidin-3- <i>O</i> -arabinoside*
R7	15.20	325	353	191	3-Caffeoyl quinic acid
R8	16.63	325	353	191, 179	Caffeoyl quinic acid
R9	17.98	280	611	431, 251	Unknown
R10	20.30	355	<b>463</b> , 301	301	Quercetin-3- <i>O</i> -glucoside
R11	21.01	325	381	191, 179	Hydroxycinnamate derivative
R12	21.92	325	515	447, <b>353</b> , 191	Dicafeoyl quinic acid

Signals characteristic of procyanidin components (e.g. at *m/z* 577, 865 and 1153 were present in the rowan sample but not in discrete peaks. \*Small amounts of another peak with PDA and *m/z* properties characteristic of cyanidin-3-*O*-galactose were also seen. Figures in bold denote the dominant signal.

buffer, enzyme, inhibitor then substrate. The substrate was added last to start the reaction. The assay was linear over for 2 h at 37 °C. Then samples were centrifuged at 16,000 rpm for 5 min and the absorbance at 410 nm read in a UV spectrophotometer. All samples were carried out in triplicate and values were presented as % control activity ± standard errors. Statistically-significant differences were assessed using Student's *T*-test.

Acarbose (Sigma Chem. Co. Ltd., product number A8980) was dissolved at 1 mg/ml in UPW. Chlorogenic acid (Sigma Chem. Co. Ltd., product number C3878) and cyanidin-3-*O*-glucoside (Extra-Synthese, Genay, France) were dissolved in UPW at suitable concentrations.

#### 2.4. Liquid chromatography–mass spectrometry (LC–MS)

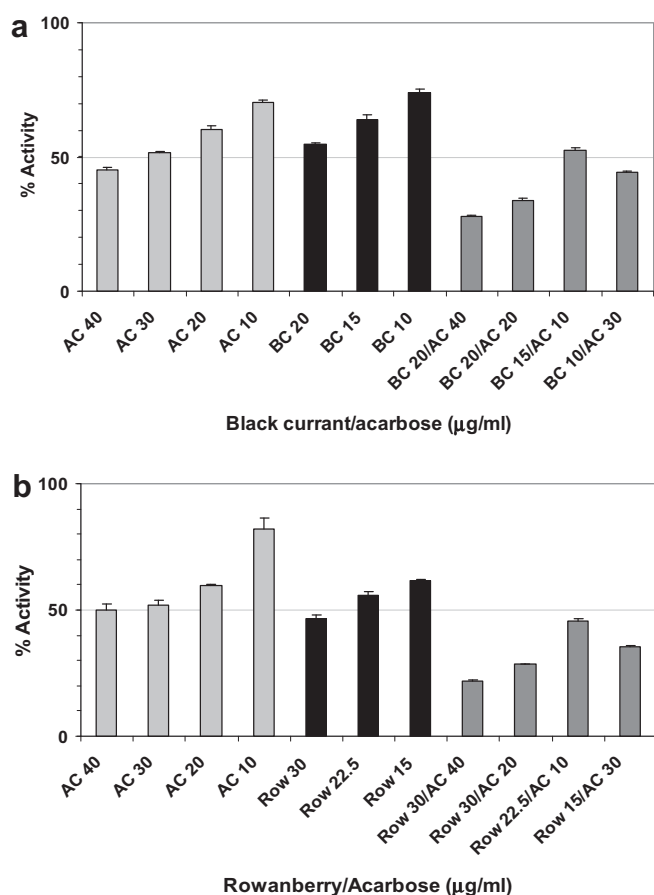
Samples (containing 20 µg GAE by Folin assay) were analysed on a LCQ-DECA system, comprising Surveyor autosampler, pump, photodiode array detector (PDAD), and a ThermoFinnigan mass spectrometer iontrap. The PDAD scanned three discrete channels at 280, 365, and 520 nm. Samples were eluted with a gradient of 5% acetonitrile (0.1% formic acid) to 40% acetonitrile (0.1% formic acid) on a C18 column (Synergi Hydro C18 with polar end capping, 4.6 mm × 150 mm, Phenomenex Ltd.) over 30 min at a rate of 200 µl/min. The LCQ-DECA liquid chromatography–mass spectrometer was fitted with an electrospray ionisation interface, and the samples were analysed in positive- and negative-ion mode.

There were two scan events: full-scan analysis followed by data-dependent MS/MS of the most intense ions. The data-dependent MS/MS used collision energies (source voltage) of 45% in wide-band activation mode. The MS detector was tuned against cyanidin-3-*O*-glucoside (positive mode) and against chlorogenic acid (negative mode). Polyphenol components were putatively identified using their PDA, MS and MS<sup>2</sup> properties using data gathered in-house and from literature.

Anthocyanins in the black currant extracts were quantified against a standard curve of cyanidin-3-*O*-glucoside. Samples were run in triplicate and the peak areas for the four main anthocyanins (cyanidin-3-*O*-glucoside, cyanidin-3-*O*-rutinoside, delphinidin-3-*O*-glucoside and delphinidin-3-*O*-rutinoside; peaks 3 and 4, Fig. 2) were selected using their [M+H] *m/z* values and calculated using the resident Xcalibur software. Chlorogenic acid derivatives in rowanberry were also quantified. Peak areas of their absorbance at 280 nm were obtained and contents estimated against a standard curve of chlorogenic acid.

### 3. Results

Black currant and rowanberry extracts were effective inhibitors of α-glucosidase with IC<sub>50</sub> values of 20 and 30 µg GAE/ml respectively (Fig. 1a and b). Acarbose inhibited α-glucosidase in a dose-dependent manner giving an IC<sub>50</sub> value of ~40 µg/ml, which is similar to previous reports (Akkarachiyasit, Charoenlertkul, Yib-



**Fig. 3.** Effect of co-incubation of berry extracts and acarbose. (a) Effect of combining black currant extracts and acarbose on  $\alpha$ -glucosidase activity. A single representative experiment is shown but the experiment was repeated three times with the same results; (b) effect of combining rowanberry extracts and acarbose on  $\alpha$ -glucosidase activity. A single representative experiment is shown but the experiment was repeated three times with the same results.

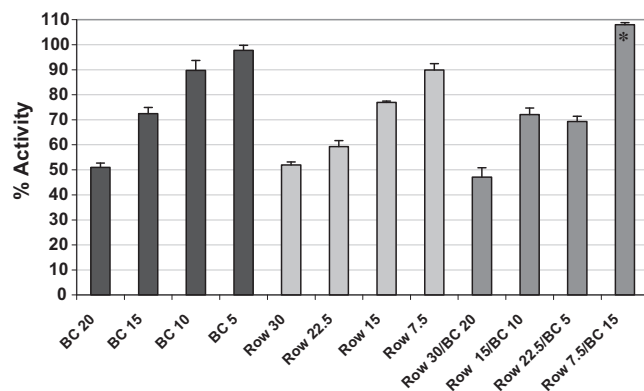
chok-anun, & Adisakwattana, 2010). Therefore, black currant and rowanberry extracts were, at least, as effective as acarbose in the same assay system.

Polyphenol-rich extracts from raspberry and cloudberry were poor inhibitors only yielding 10–20% inhibition at 100  $\mu$ g GAE/ml. No  $IC_{50}$  value could be calculated but it was substantially greater than 200  $\mu$ g/ml (Fig. 1d and e). These results are broadly supported by previous work (McDougall et al., 2005) and the screening of berry extracts presented before (Whitson et al., 2010).

The low level of inhibition by the rowanberry proanthocyanidin-rich fraction compared to the whole rowanberry extract (compare Fig. 1b and c) is converse to the situation with amylase inhibition by berry extracts (Grussu et al., 2011) and suggests that these tannin components are not influential in inhibition of  $\alpha$ -glucosidase.

Given their polyphenol compositions (McDougall, Martinussen, & Stewart, 2008), the lack of inhibition by raspberry and cloudberry strongly suggests that, like the proanthocyanidins, ellagitannins are poor inhibitors of  $\alpha$ -glucosidase. This is also supported by previous work that indicated ellagitannin-rich fractions from raspberry were ineffective against  $\alpha$ -glucosidase (McDougall et al., 2005).

The black currant extracts were enriched in anthocyanins (Fig. 2; compare traces a and b; Table 2). In fact, the composition of black currant extracts was similar to published accounts (e.g. Slimestad & Solheim, 2002). They were enriched in anthocyanins



**Fig. 4.** Effect of co-incubation of black currant and rowanberry extracts. A single representative experiment is shown but the experiment was repeated three times with similar results. The asterisk denotes where the % activity values were significantly different (by *T*-test) from that of the highest concentration of berry extract alone used.

(especially the characteristic glucosides and rutinosides of cyanidin and delphinidin) which comprised approximately 70% content by peak area with smaller amounts of flavonols and hydroxycinnamate derivatives.

The rowanberry extracts were characterised by the presence of chlorogenic acids, other hydroxycinnamate derivatives and substantial amounts of flavonol derivatives (Fig. 2, trace C and Table 1). The anthocyanin content of the rowanberry extract was low, possibly due to the variety used (Hukkanen, Pölonen, Kärenlampi, & Kokko, 2006) or the extraction method applied. However, the overall composition was similar to previous reports (Kylli et al., 2010). The three main chlorogenic acids in the rowanberry extract (peaks R2, R7 and R8) accounted for approximately 65% of the total phenol content (results not shown).

Chlorogenic acid was tested and was found to yield an  $IC_{50}$  value of 300  $\mu$ g/ml, which was ten-times higher than the black currant or rowanberry extracts. Cyanidin 3-*O*-glucoside was also tested and yielded an  $IC_{50}$  value of 205  $\mu$ g/ml for  $\alpha$ -glucosidase inhibition (results not shown). Presenting both these compounds at their respective  $IC_{50}$  values (i.e. 100:100) caused greater inhibition (~33% control activity) which indicated a synergistic effect. Indeed, presenting these components at half their  $IC_{50}$  values (50:50) also proved synergistic with inhibition at ~55% (results not shown).

Both rowanberry and black currant extracts potentiated the inhibition of  $\alpha$ -glucosidase caused by acarbose (Fig. 3a and b). This was demonstrated by the increased inhibition caused by combining acarbose at its  $IC_{50}$  value with the berry extracts at their  $IC_{50}$  values. Moreover, combination of berry extracts at 75% of their  $IC_{50}$  values could replace the inhibition “lost” by reducing the acarbose levels to 25%  $IC_{50}$  value. In both cases, addition of rowanberry or black currant extracts returned inhibition to around 50% of control activity. Overall, these results suggest that acarbose and the berry extracts can act in a synergistic manner to inhibit  $\alpha$ -glucosidase *in vitro*.

On the other hand, when black currant and rowanberry extracts were added together (Fig. 4) in various combinations, there was no obvious increase in inhibition over the levels achieved by the individual extracts. Only the combination of the black currant and rowan extracts at their  $IC_{50}$  values (20 and 30  $\mu$ g/ml, respectively) appeared to increase inhibition but this was not statistically significant as assessed by *T*-test. However, the combination of 7.5  $\mu$ g rowanberry and 15  $\mu$ g black currant extracts was less effective than either the black currant extract at 15  $\mu$ g/ml or the rowanberry extract at 7.5  $\mu$ g/ml alone. This confirms that there was no additive



or synergistic effect between the black currant and rowanberry polyphenols and, indeed, suggests that the berry components may actually act in an antagonistic fashion.

#### 4. Discussion

The finding that polyphenol-enriched extracts from certain berries can inhibit  $\alpha$ -glucosidase *in vitro* confirms and extends previous reports (McDougall et al., 2005; McDougall et al., 2008; Whitson et al. 2010). It was notable that the black currant and rowanberry extracts were more effective than acarbose in the same assay system. A recent study also suggested that polyphenol-rich extracts of high bush blueberries were also more effective than acarbose (Johnson, Lucius, Meyer, & Gonzalez de Mejia, 2011).

The low levels of inhibition by cloudberry and raspberry strongly suggest that ellagitannins were not effective inhibitors. Inhibition of  $\alpha$ -amylase activity by ellagitannins in raspberry and by proanthocyanidins in rowanberry was associated with the formation of enzyme-tannin complexes (Grussu et al., 2011; McDougall et al., 2005) which prevented the enzyme from interacting with starch. However as  $\alpha$ -glucosidase was less susceptible to inhibition by purified proanthocyanidins (PACs) from rowanberry than the whole rowanberry extract, this simple protein-binding mechanism may be less important for inhibition of  $\alpha$ -glucosidase. However, where they make up a major part of the polyphenol composition, PACs may influence glucosidase inhibition more strongly (e.g. Adisakwattana, Lerdsuwankij, Poputtachai, Ubonwan, & Aukkrapon, 2010).

The most active berry extracts, from rowanberry and black currants, have very different phenolic compositions. The black currant extract was enriched in anthocyanins but also contains appreciable amounts of flavonols and hydroxycinnamic acid (HCA) derivatives. Anthocyanins have been implicated in the inhibition of  $\alpha$ -glucosidase by berry extracts (e.g. McDougall et al., 2005; You, Feng, Xi, Pengju, & Jiang, 2011) and in the inhibition by sweet potato extracts (Matsui et al., 2002). It is intriguing to postulate that the glycosylated anthocyanins may act as substrate mimics and competitively interfere with hydrolysis of the substrate. Indeed, cyanidin-3-*O*-galactose was approximately three times more effective than the aglycone cyanidin in inhibiting rat intestinal sucrase activity (Akkarachiyasit et al., 2010) but cyanidin was found to be more effective than cyanidin-3,5-diglucoside by other researchers (You et al., 2011).

Rowanberry extracts contained mainly HCA derivatives, especially chlorogenic acids (CGAs), but had low anthocyanin content (Kylli et al., 2010). Some proanthocyanidins were present in the rowanberry extract but their contribution to inhibition by the whole extract may be limited. Chlorogenic acids inhibit  $\alpha$ -glucosidase and have been implicated as causal agents in the anti-hyperglycaemic effects of coffee extracts (Tateishi, Han, & Okuda, 2004). Indeed, some additive effects were noted in combining cyanidin-3-*O*-glucoside and chlorogenic acid but the effects were noted at much higher concentrations than with the berry extracts. For example, rowanberry extracts were 10 times more effective than pure CGA and the black currant extracts were also ten-times more effective than pure cyanidin-3-*O*-glucoside [which gave an  $IC_{50}$  of  $\sim 205 \mu\text{g/ml}$  or  $\sim 440 \mu\text{M}$ ; similar to the  $IC_{50}$  previously reported Akkarachiyasit et al., 2010]. In addition, we found that a purified anthocyanin preparation from black currants (an essentially pure mixture of cyanidin and delphinidin glucosides and rutosides) was no more effective than cyanidin-3-*O*-glucoside alone (results not shown).

The more potent inhibition by whole berry extracts suggests that other components present in the berry extracts enhance inhibition. Rowanberry contained, at least, one dicaffeoylquinic acid

derivative (Fig. 2; Table 1, peak R12) and such components have been demonstrated to be  $\alpha$ -glucosidase inhibitors (Kamitani, Iwai, Fukunaga, Kimura, & Nakagiri, 2009). Rowanberry also contained substantial amounts of flavonol glycosides (e.g. quercetin glucoside; peak R10, Fig. 2 and Table 1). Therefore, it is also feasible that interactions between flavonol + HCA derivatives potentiated inhibition. Indeed, flavonol glycosides have been shown to inhibit  $\alpha$ -glucosidase *in vitro* (Habtemariam, 2011; Jo et al., 2011; Yoshida, Hishida, Iida, Hosokawa, & Kawabata, 2008) and differences in effectiveness between various flavonol glycoside structures have been reported (e.g. Phuwapraisirisan, Puksasook, Kokpol, & Suwanborirux, 2009). Black currants also contain a range of flavonol derivatives, albeit in lesser amounts than anthocyanins, which could act in concert with the more abundant anthocyanins. Future work in this area could extend the study of inhibition of  $\alpha$ -glucosidase with combinations of CGAs and anthocyanins by looking at the effects of purified flavonols.

The potent inhibition by the chlorogenic acid-rich rowanberry and the anthocyanin-rich black currant extracts suggests that  $\alpha$ -glucosidase can be inhibited by a range of dietary-derived polyphenolic components. Indeed, inhibition of  $\alpha$ -glucosidase has been reported for a wide range of polyphenol-rich plant extracts and purified components from a range of phenolic classes (e.g. Kumar, Narwal, Kumar, & Prakash, 2011; McDougall et al., 2008). This somewhat promiscuous inhibition allied to a lack of detail about dose-effectiveness or the nature of the phenolic composition of extracts has made comparison and identification of quality-structure activity relationships difficult. Use of different  $\alpha$ -glucosidase preparations from different sources and assay conditions has also complicated comparisons. This situation may be improved by moves to screen berry germplasm for  $\alpha$ -glucosidase inhibition (e.g. Johnson et al., 2011) but only if the phytochemical composition of the inhibitory accessions are examined.

That the black currant and rowanberry extracts, which were very effective individually, did not produce an additive effect when added together was unexpected. The lack of an additive effect suggests that phenolic components in one extract may have prevented components in the other extract from causing inhibition i.e. they acted in an antagonistic fashion. Theoretically, this could be due to the higher affinity of one component for the same binding site (perhaps the active site) or one component binding to a site other than the active site which influenced the affinity of the active site for the other inhibitory component. Understanding the nature of this competition requires further studies with purified components and studies on the effect on the order of addition of components.

CGA and derivatives (Matsui et al., 2004) and finger millet phenolics (Shobana, Sreerama, & Malleshi, 2009) inhibited  $\alpha$ -glucosidase in a non-competitive mode, which often involves binding at a site other than the active site (Fersht, 1985). This could explain the synergy of the berry polyphenols with acarbose which targets the active site. If phenolic components from black currant and rowanberry were competing for this secondary site, this may explain their antagonism. Cyanidin-3-*O*-glycosides can inhibit  $\alpha$ -glucosidase *in vitro* (Akkarachiyasit et al., 2010) but their mechanism of action is still not known. Nevertheless, it is intriguing that cyanidin-3-*O*-rutoside inhibits pancreatic amylase (Adisakwattana, Yibchok-Anun, Charoenlertkul, & Wongsasiripat, 2011) in a mixed type manner, a combination of both competitive and non-competitive modes (Fersht, 1985), which may arise through binding at the active site but also at another site on the enzyme. Future work could focus on the identification of potential different binding sites for polyphenol components on  $\alpha$ -glucosidase through the use of modeling studies (e.g. Rasttija, Beslo, & Nikolic, 2011).

The combination of acarbose and phenolic-rich berry extracts showed additive effects thus suggesting that such polyphenols could substitute for or be used in conjunction with pharmaceutical

agents in maintaining glycaemic control, which may be useful in the treatment of type 2 diabetes. Additive effects between acarbose and polyphenols in inhibiting glucosidase have been noted previously (e.g. Adisakwattana et al., 2011). Lowering the required dose of acarbose may reduce its potential side effects (Philippe & Raccach, 2009). In addition, identifying natural components with high  $\alpha$ -glucosidase but lower  $\alpha$ -amylase inhibitory potential, such as the black currant extracts, could prevent certain side-effects of acarbose, which are largely due to undigested, but readily-fermented, starch reaching the colon (Rosenstock et al., 1998).

All-in-all, these results confirm that  $\alpha$ -glucosidase is susceptible to inhibition by a range of phenolic derivatives *in vitro* (McDougall et al., 2008). The effectiveness of inhibition by different individual phenolic components depends on their site of action, their mechanism and their binding affinities. Synergies and antagonisms between the different phenolic components may potentiate or reduce inhibition. Importantly, these berry extracts inhibit  $\alpha$ -glucosidase at concentrations (<50  $\mu$ g GAE/ml) which could be easily reached in the GIT after intake of berries or juices (Kahle et al., 2006). Therefore, these components would be present at relevant physiological concentrations and in the correct location to be effective after intake of berries or berry juices. Many studies have already shown that intake of plant material rich in polyphenols can cause anti-hyperglycaemic effects in animals (e.g. Hogan et al., 2010; Jo et al., 2011; Takikawa, Inoue, Horio, & Tsuda, 2010), possibly via  $\alpha$ -glucosidase and/or  $\alpha$ -amylase inhibition. However, dietary phenolic compounds may also interfere with glucose uptake at the brush border interface (Welsch, Lachance, & Wasserman, 1989). Indeed, phenolic-rich berry extracts have been shown to modulate glycaemic responses in humans (e.g. Torronen et al., 2010). However, other studies have found berry intake less effective (e.g. Clegg, Pratt, Meade, & Henry, 2011). It is possible that differences in the form (berries vs. juices) or the total polyphenol dose (and composition) ingested can explain these contradictory results but it is clear that further human intervention studies are warranted. In addition, intake of nutraceuticals or tailored products based on polyphenol-rich plant or berry extracts may also yield benefits due to their proposed beneficial underpinning of endogenous antioxidant systems, which have also been implicated in ameliorating other conditions relevant to type 2 diabetes (e.g. Rodrigo, Miranda, & Vergara, 2011).

In summary, the inhibitory activity of the berry-derived polyphenols towards  $\alpha$ -glucosidase offers the potential for type 2 diabetics to manage their own glycaemic control via dietary means. Once the “best” inhibitors are identified, they could be purified and formulated into drug-like treatments. Alternatively, such information could be used to design juice blends or, in the longer term, the most active compounds could also be identified as targets for enhancement in soft fruit breeding programmes to provide bioactive-enhanced fruit.

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