

Berry Polyphenols Inhibit α -Amylase *in Vitro*: Identifying Active Components in Rowanberry and Raspberry

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 Supporting Information

ABSTRACT: Polyphenol-rich extracts from a range of berries inhibited α -amylase *in vitro*, but the most effective were from raspberry and rowanberry (IC_{50} values of 21.0 and 4.5 μ g/mL, respectively). The inhibitory components were examined by different approaches. Extracts from yellow and red raspberries were equally able to inhibit α -amylase. Because the yellow raspberry extracts effectively lacked anthocyanins, this suggested that they were not crucial for amylase inhibition. Notably, however, higher levels of other phenolic components in yellow raspberries (particularly, ellagitannins) did not increase amylase inhibition. Amylase inhibition in rowanberry was recovered in a fraction enriched in proanthocyanidins (PACs). Inhibition was ameliorated by bovine serum albumin, suggesting that PACs acted by binding to amylase. Co-incubation of rowanberry PACs with acarbose reduced the concentration of acarbose required for effective amylase inhibition. Such synergistic interactions could have implications for the current clinical use of acarbose for postprandial glycaemic control in type-2 diabetics.

KEYWORDS: Amylase, berry, diabetes, glycaemic response, polyphenols, starch

■ INTRODUCTION

Over the past decade, the theory that the health benefits associated with a diet rich in fruits and vegetables may be derived, in part, from the intake of natural antioxidants¹ has gained popularity. The main antioxidants in fruits are vitamin C and polyphenols.² The polyphenols are a diverse range of chemical classes that share the ability to act as chain-breaking antioxidants, which are proposed to protect against the damage caused by free radicals to DNA, membrane, and cellular components.³ However, it is becoming clear that different classes of polyphenol compounds differ greatly in their bioavailability.⁴ Components, such as anthocyanins, which are abundant in berries,⁵ have low serum bioavailability and/or poor stability under serum conditions *in vivo*⁶ and are therefore unlikely to provide protection at the cellular level. The major part of the ingested polyphenol dose from berries is not taken up into the circulation and passes through the upper gastrointestinal tract (GIT) to the large intestine, where they may be biotransformed or broken down by the indigenous microbiota.⁷ As a result, the health benefits derived from a diet rich in polyphenol antioxidants may be partly delivered through effects carried out within the GIT. Polyphenols or their metabolites may influence health by preventing damage caused by free radicals generated in the GIT from foods,⁸ modulating intestinal inflammatory responses,⁹ influencing the development of cancers in intestinal cells in direct contact with the gut contents,¹⁰ or beneficially modulating the colonic microbiota.¹¹ In addition, it has become clear that polyphenols may be able to modulate nutrient availability through the inhibition of digestive enzymes involved in lipid and starch breakdown, which could lead to beneficial effects on calorie intake and obesity¹² and blood glucose control,¹³ respectively. In

previous work, we observed that polyphenol-rich extracts from berries could inhibit the two main enzymes involved in starch digestion, α -amylase and α -glucosidase, *in vitro* but at levels easily achievable in the GIT.¹⁴ Indeed, inhibition of these enzymes is the target of drugs, such as acarbose (known as Glucobay and Precose), which are used therapeutically to control blood glucose levels in type-2 diabetics after starch-containing meals.¹⁵ Since this work, other groups have confirmed the ability of berry extracts to inhibit these enzymes *in vitro* and some have begun to rank cultivars on this potential health benefit.¹⁶

In this study, we focused on the ability of polyphenol-rich extracts from a range of berries to inhibit α -amylase *in vitro*. Using a range of berry extracts with characteristic and different polyphenol compositions, we hope to identify candidate components responsible for the inhibition. We used two different approaches to identify the active inhibitory components in the two most effective berry extracts. We have examined the possibility that berry polyphenols can act in concert with the therapeutically used α -amylase inhibitor, acarbose, to reduce the dose required for effective glycaemic control.

■ MATERIALS AND METHODS

Plant Material and Extraction. Most fruits were obtained in the summer of 2008. Black currants (*Ribes nigrum* L. variety 8982-6) were obtained from Bradenham Hall, Norfolk, U.K., and blueberries (*Vaccinium corymbosum*, variety Berkeley) were grown at SCRI.

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Cloudberries (*Rubus chamaemorus*), Arctic bramble (*Rubus stellatus* × *Rubus arcticus*), lingonberries (*Vaccinium vitis-idaea*), and rowanberries (*Sorbus aucuparia*, variety Sahharnaja) were obtained from Dr. Harri Kokko, University of Kuopio, Finland. Strawberries (*Fragaria ananassa*, variety Elsanta) and raspberries (*Rubus idaeus*, variety Glen Ample) were obtained from local farmers. All fruits were picked at full ripeness, frozen within a day of picking, and then, where required, transported frozen to SCRI. Blackberries (*Rubus fruticosus* L.), pomegranates (*Punica granatum* L.), and red wine (Echo Falls, a Merlot variety wine from Mission Bell Winery, Madera, CA) were purchased from a local supermarket. Red wine was concentrated by rotary evaporation to remove alcohol.

Yellow raspberries (SCRI accession number 97134B1) were obtained from the SCRI breeding program in the summer of 2009 as a kind gift from Dr. Nikki Jennings. Fruits were extracted by the protocol outlined previously.¹⁷ Briefly, the frozen fruits were homogenized in an equal volume to weight of 0.2% (v/v) formic acid in ultra-pure water (UPW) using a Waring blender (5 times for 15 s on full power). The extracts were filtered through tripled muslin and then centrifuged at 4000 rpm for 10 min at 4 °C to remove suspended polysaccharides and pulp. All extracts were applied to individual C18 solid-phase extraction (SPE) units (Strata C18-E, GIGA units, 10 g capacity; Phenomenex, Ltd., Macclesfield, U.K.) prewashed in 0.2% (v/v) formic acid in acetonitrile and then pre-equilibrated in 0.2% (v/v) formic acid in water. The unbound material, which contained the free sugars, organic acids, and vitamin C, was collected. The SPE units were washed with a unit volume of 0.2% (v/v) aqueous formic acid and then with 2 volumes of UPW. The polyphenol-enriched bound extracts eluted with 80% acetonitrile/UPW. These C18-bound extracts were evaporated to dryness in a SpeedVac (Thermo Scientific, Waltham, MA).

Fractionation on Sephadex LH-20. Sorption to Sephadex LH-20 in aqueous ethanol and stepwise elution with solutions of aqueous ethanol and acetone are an established method for polyphenol components. The method was adapted from the *Tannins Handbook* (kindly made available at www.users.muohio.edu/hagermae/tannin.pdf). Briefly, a column of Sephadex LH-20 was washed in 80% (v/v) ethanol/water and then 80% (v/v) acetone/water before being equilibrated with three volumes of 5% ethanol. Rowanberries were extracted in 50% ethanol/water as above and then rotary-evaporated to remove ethanol. After filtration through Whatman GF/C paper, the rowanberry extract was diluted 1:1 with 10% ethanol. This extract was applied to the column, and various fractions were collected (see Figure S1 of the Supporting Information). Fractions 1 and 2 were eluted as unbound run through and by further application of 5% ethanol, respectively. Fractions 3–5 were eluted successively by application of 25% ethanol, and fraction 6 was eluted with 50% ethanol. Fractions 7 and 8 were eluted with 50% acetone, and fraction 9 was eluted using 80% acetone.

Anthocyanin and Phenol Assays. The total anthocyanin concentration was estimated by a pH differential absorbance method.¹⁸ The absorbance value was related to the anthocyanin content using the molar extinction coefficient calculated for cyanidin-3-O-glucoside (purchased from ExtraSynthese, Ltd., Genay, France). The phenol content was measured using a modified Folin–Ciocalteu method.¹⁸ Phenol contents were estimated from a standard curve of gallic acid. Samples were dried in aliquots to a constant phenol content using a SpeedVac.

Amylase Assay. This assay was conducted as described previously.¹⁴ Briefly, stock starch solution was prepared by suspending 1% (w/v) soluble potato starch (Sigma Chem. Co., Ltd., product S-2360) in synthetic saliva buffer and gelatinizing the mixture for 15 min at 90 °C. Porcine pancreatic α -amylase (Sigma Chem. Co., Ltd., product A-3176) was dissolved in synthetic saliva buffer at 380 mg/L. The control assay contained 800 μ L of synthetic saliva buffer, 100 μ L of α -amylase, and 100 μ L of UPW or extract, and the reaction was started by the addition of 500 μ L of starch solution. The positive extract assays contained various amounts of extracts in the 100 μ L volume. To estimate

IC_{50} values (the amount of phenols that gave 50% inhibition of amylase), assays were carried out with a range of phenol contents. Acarbose (Sigma Chem. Co. Ltd., product number A8980) was dissolved at 1 mg/mL in UPW. Bovine serum albumin (BSA, Sigma Chem. Co. Ltd., product number A4503) was dissolved at 5 mg/mL in UPW.

Assay for Reducing Termini Using PAHBAH. A 5% (w/v) stock solution of *p*-hydroxybenzoic acid hydrazide (PAHBAH) in 0.5 M HCl was diluted 1:4 with 0.5 M NaOH to give the working PAHBAH reagent. Triplicate samples (50 μ L) of assays were taken at fixed times and added to 1 mL of PAHBAH reagent in a 1.5 mL tube. After heating for 10 min at 100 °C, the absorbance at 410 nm was measured. Controls lacking enzyme were used as blanks. An assay time of 5 min was taken as the standard because the rate of production of reducing termini was linear up to this point. The percent of control amylase activity was calculated as the difference between the control and the plus extract reactions divided by the control reaction.

Liquid Chromatography–Mass Spectrometry (LC–MS). Samples (containing 20 μ g of gallic acid equivalents by the Folin assay) were analyzed on a LCQ-DECA system, comprising a Surveyor auto-sampler, pump, photodiode array detector (PDAD), and a Thermo-Finnigan mass spectrometer ion trap. 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 × 150 mm, Phenomenex, Ltd.) over 60 min at a rate of 400 μ L/min. The LCQ-DECA liquid chromatography–mass spectrometer was fitted with an electrospray ionization interface, and the samples were analyzed 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 ellagic acid (negative mode). Polyphenol components were detected and identified using their PDA, MS, and MS^2 properties using data gathered in-house and from the literature (raspberry^{10,19–22} and rowanberry²³). The relative content of polyphenols in yellow and red raspberry extracts was determined by comparing the peak areas for triplicate samples using the Xcalibur software (Thermo, Ltd., U.K.).

■ RESULTS

A range of polyphenol-rich extracts from berries inhibited α -amylase activity *in vitro* (Figure 1) with various levels of effectiveness. The order of effectiveness was largely maintained when re-assayed at lower concentrations and indicated that extracts from raspberries and rowanberries were particularly effective. Indeed, the rowanberry and raspberry extract gave IC_{50} values of 4.5 and 21.0 μ g of gallic acid equivalent (GAE)/mL, respectively, suggesting that they contained potent inhibitors.

The identity of the α -amylase inhibitors in raspberry and rowanberry extracts was examined by two different approaches. First, extracts were prepared from a red raspberry (Glen Ample) and from a yellow raspberry accession from SCRI's breeding program and then tested for their ability to inhibit amylase. Both the polyphenol-rich extracts caused complete inhibition of activity at 50 μ g/mL, and the red raspberry extract gave an IC_{50} value of 13.5 μ g/mL, which is slightly lower than the original raspberry extract. However, the yellow extracts gave an IC_{50} value of ~16.5 μ g/mL, which is essentially similar to the red extracts (Figure 2).

As could be expected, the yellow raspberries effectively lacked anthocyanins and had approximately 25-fold lower total anthocyanin content (results not shown) than the red raspberry

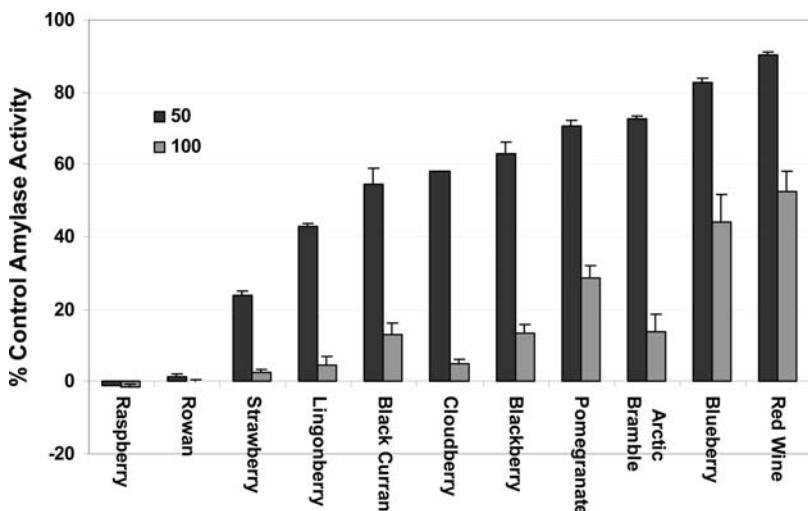


Figure 1. Inhibition of α -amylase activity *in vitro* by berry extracts. Extracts were assayed at 50 and 100 μ g of GAE/mL. The percent control activity was assessed for each set of assays. The values shown are averages of triplicate assays \pm standard error.

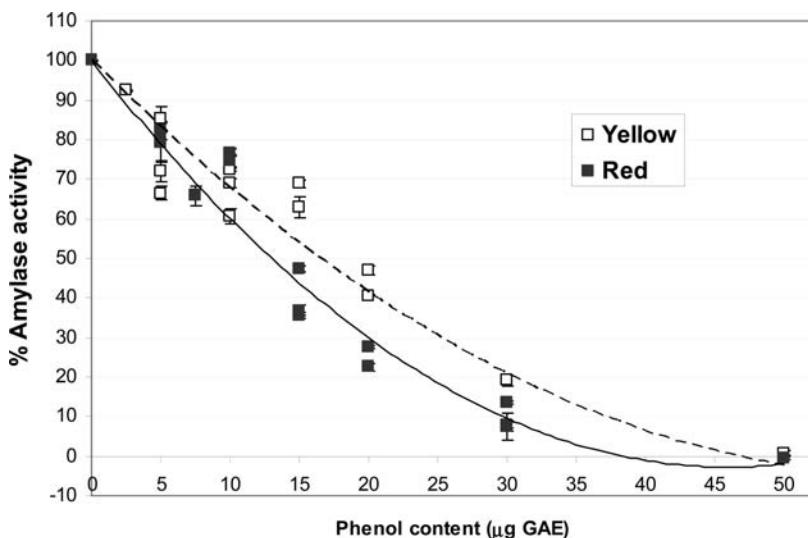


Figure 2. Amylase inhibition by extracts from red and yellow raspberries. Extracts were assayed at a range of phenol contents. The percent control activity was assessed for each set of assays. The values shown are averages of triplicate assays \pm standard error. The trend lines were fitted using Excel software.

extracts. However, the yellow extracts had a higher content of other polyphenol components, including ellagittannins, flavonols, and hydroxycinnamate derivatives (Figure 3 and Table S1 of the Supporting Information) as assessed by LC-MS. However, there was a general increase in the levels of non-anthocyanin components, but there were differences within polyphenol groups. For example, the relative content of the ellagittannin, Lambertian C, was increased 7-fold over the red raspberry, but Sanguin H6 was only increased 4-fold. Also, within the flavonols, the relative content of quercetin-3-glucuronide was increased over 7-fold, whereas the levels of quercetin rutinosides were effectively similar.

Therefore, the absence of anthocyanins did not markedly reduce amylase inhibition, and the presence of relatively higher amounts of ellagittannins did not substantially improve the inhibition of amylase. This suggests that ellagittannins are not solely responsible for amylase inhibition. This confirms the

finding of the screening process in which raspberry extracts were more effective inhibitors than cloudberry extracts, which have similar polyphenol composition, except for the very low levels of anthocyanins in cloudberry extracts.⁵

In another approach, the rowanberry extracts were fractionated using step elution chromatography on Sephadex LH-20 (see Figure S1 of the Supporting Information). The fractions were obtained by elution with increasingly less polar solvents, and the main constituents were identified by LC-MS. The original rowanberry extract had a similar composition to previous work²³ containing hydroxycinnamic acids (mainly chlorogenic acids), flavonols, and anthocyanins. The fractionation of the main components could be readily followed. Fraction 1 was mainly composed of chlorogenic acids. Fractions 2 and 3 contained chlorogenic acids and anthocyanins in varying amounts. Fraction 4 contained mainly quercetin hexoses. Fraction 5 contained as yet unidentified phenolic components.

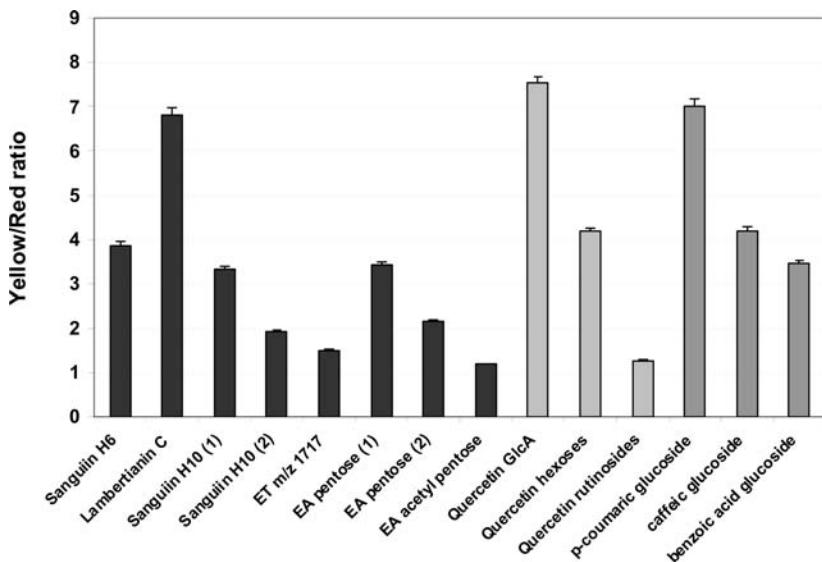


Figure 3. Relative contents of selected phenolic components in yellow and red raspberry extracts. Contents were estimated from peak areas at m/z values characteristic of the various components and calculated using Xcalibur software. Further details are available in Table S1 of the Supporting Information. Each value represents the mean of peak areas from triplicate injections \pm standard error. The peak areas for the yellow raspberry extracts are expressed as ratios of the peak areas for the red raspberry extracts. The component with $[M - H] = 1717$ was identified as an ellagitannin equivalent to Sanguin H6 minus a gallic acid group.²⁰ GlcA denotes a glucuronide group.

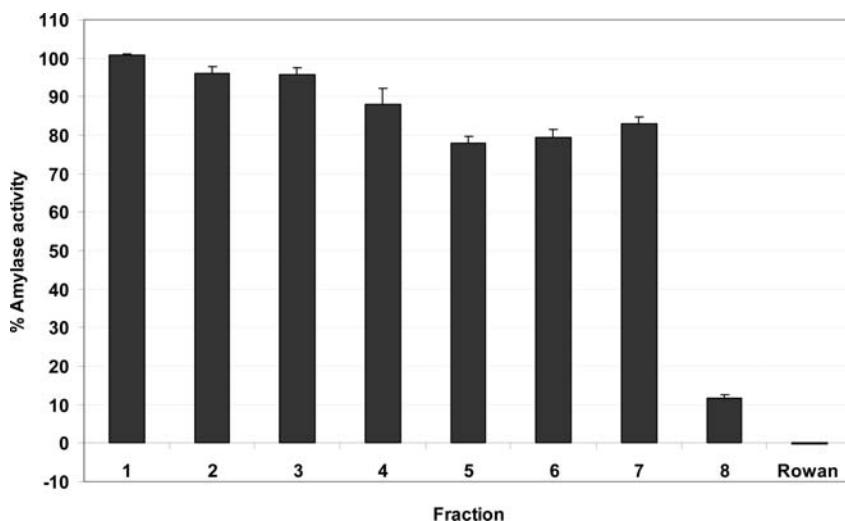


Figure 4. Amylose inhibition by rowanberry fractions separated by Sephadex LH-20. The percent control activity was assessed for each set of assays. The values shown are averages of triplicate assays \pm standard error, with each fraction added at 20 μ g of GAE/mL. The whole rowanberry extract was assayed as a positive control.

Fraction 6 contained low levels of unidentified phenolics. Fraction 7 mainly contained quercetin coumaroyl hexoses, and fraction 8 was mainly composed of proanthocyanidins (PACs) (Figure 4). As noted previously,¹⁷ PACs do not separate well on reverse-phase LC but the fraction gave an MS spectra characteristic of A- and B-type PACs composed of epicatechin units (Figure 5B²⁴) known to be present in rowanberry.^{23,25} For example, there are signals at both 1151 and 1153, which are characteristic of the A- and B-type tetramer of epicatechin, respectively.

Only the PAC-rich fraction 8 caused substantial inhibition of amylase at 20 μ g/mL (Figure 4). Indeed, this fraction gave an IC_{50} value of ~ 5 μ g/mL for amylase inhibition compared to 4.5 μ g/mL for the unfractionated rowanberry extract. Therefore, the

purified rowanberry PACs were as effective as the whole rowanberry extract, even though the concentration of PACs in the whole rowanberry extract must have been considerably lower than that in the PAC-enriched fraction.

The addition of BSA was protective against inhibition by PACs (Figure 6), which suggests that PACs may operate by binding to amylase and preventing interaction with the starch substrate, as suggested previously.¹⁴ However, the protective effect was maximal at 100 μ g/mL when the assay contained 28.5 μ g/mL amylase (and 4.5 μ g/mL PACs), with no increase in protective effect at up to 250 μ g/mL BSA. A slight but significant increase in the control amylase activity with BSA addition suggested that part of the apparent protection against inhibition by PACs may have been due to improved amylase activity.

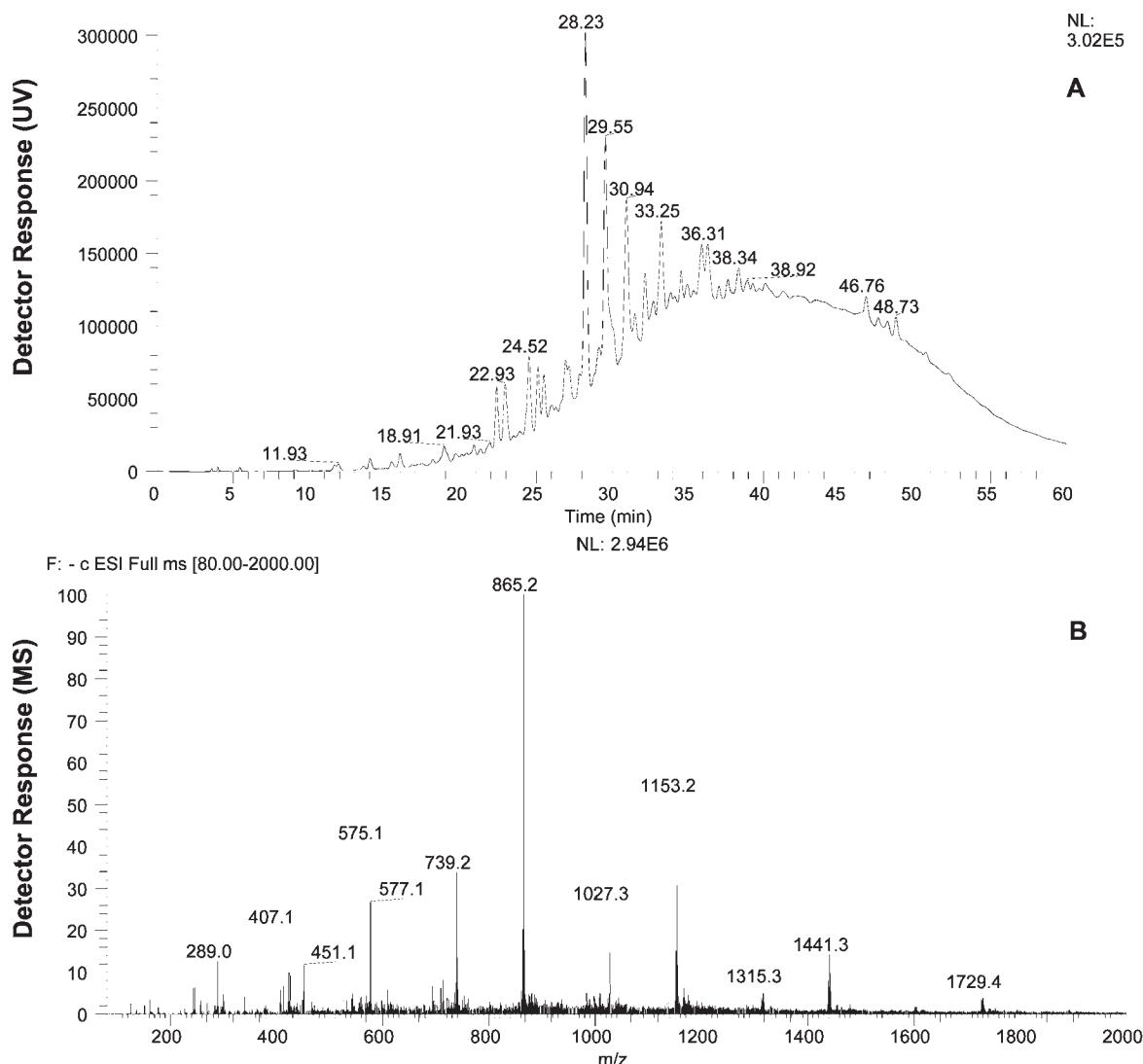


Figure 5. LC-MS trace and MS spectra of rowanberry fraction 8. (A) UV trace (at 280 nm) of rowanberry fraction 8. (B) MS spectrum for the same sample from 10 to 60 min. The values in the top right corner represent the full scale.

If the order of addition of components was changed and the reaction started by the addition of the amylase rather than the starch, then the rowanberry PACs were less effective. Not preincubating amylase with PACs reduced inhibition at the original IC_{50} value from $\sim 50\%$ to around 70% control activity (see Figure S2 of the Supporting Information). This suggests that PACs interact directly with the amylase.

Assays were carried out to assess the effects of co-incubation of rowanberry PACs with the known α -amylase inhibitor acarbose.²⁶ The IC_{50} value for acarbose was determined in the *in vitro* assay system, and it was confirmed at 0.8 $\mu\text{g}/\text{mL}$ or 1.24 μM , similar to previous reports.²⁷ The effect of PACs and acarbose were tested at various combinations of their IC_{50} values. Combining PACs and acarbose at 100% of their IC_{50} values expectedly gave considerable inhibition around 15% control activity (Figure 7). Combining them at 50% of their individual IC_{50} values gave inhibition lower than 50% control activity ($\sim 30\%$ control), which suggests some additive or synergistic effects between the two treatments. Adding them at 75% of the IC_{50} value for PACs and 25% of the IC_{50} value for acarbose gave greater inhibition than the 50:50 addition or, indeed, the

inhibition caused by the addition of 75% acarbose/25% PACs. Taken together, this indicates that the combination with PACs could reduce the effective dose of acarbose required for inhibition.

■ DISCUSSION

This study confirms previous work¹⁴ and recent studies¹⁶ that polyphenol-rich extracts from berries can inhibit α -amylase *in vitro* at low concentrations. Previous work strongly suggested that ellagitannins (ETs) in raspberry were the main active components for amylase inhibition.¹⁴ Purified ETs from strawberry have also been shown to have amylase inhibitory activity.²⁸ However, red raspberry extracts were considerably more effective than cloudberry extracts, despite the higher relative content of ETs in cloudberry extracts.⁵ Therefore, on this basis, one could propose that the presence of anthocyanins potentiated amylase inhibition by ETs. However, yellow raspberry extracts, which were relatively enriched in ETs, were no more effective than red raspberry extracts. However, other studies have suggested that red raspberries were more effective amylase inhibitors than

yellow raspberries.²⁹ In addition, the finding that red currants were more effective in inhibiting amylase than black currants,³⁰ which generally have higher anthocyanin content, does not suggest that anthocyanins play a major role in the inhibition of amylase.

Amylase inhibition was mainly recovered in a fraction enriched in PACs after fractionation of rowanberry extracts on Sephadex LH-20, and PACs have been identified as potent inhibitors of amylase in previous studies.^{31,32} The MS results suggested that the rowanberry PACs were mainly of a low degree of polymerization, but more detailed structural characterization would be useful.³³ Although the rowanberry PACs were as potent inhibitors as the whole rowanberry extract (with IC_{50} values $\sim 5 \mu\text{g/mL}$), it is clear that this fraction must have been, at least, 10-fold enriched in PACs compared to the original rowanberry extract. This suggests that the presence of the other non-PAC components may have influenced amylase inhibition. Synergism

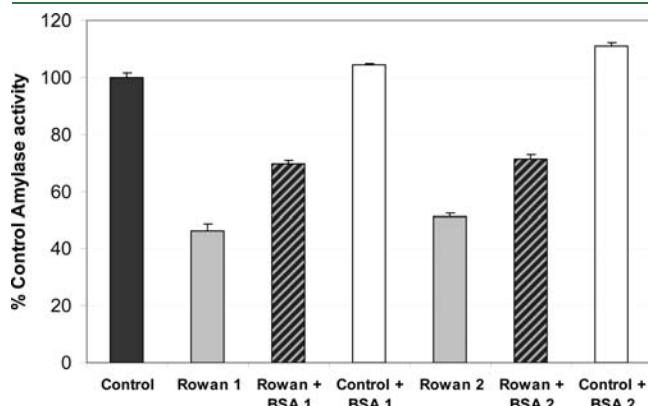


Figure 6. Effect of BSA on the inhibition of amylase by rowanberry PACs. The percent control activity was assessed for each set of assays. The values shown are averages of triplicate assays \pm standard error. Two repeat experiments are shown. Rowanberry PACs were added at $3.5 \mu\text{g}$ of GAE/mL, and BSA was added at $100 \mu\text{g}/\text{mL}$. Control assays without PACs but with BSA were run to assess any protective effect of BSA on amylase activity.

of components may also partly explain the great difference in effectiveness between the rowanberry extracts and the lingonberry extracts, which also contain PACs but differ considerably in their polyphenolic composition.^{5,17,33}

The protection of amylase inhibition by BSA and the order of addition studies suggest that PACs influence amylase activity through their protein-binding activity, as suggested previously,³⁴ and reduce the ability of the enzyme to interact with the starch. Given their astringency, it seems very likely that ETs also operate by this mechanism.³⁵ Therefore, although ETs in raspberry and PACs in rowanberry obviously contribute greatly to the inhibition of amylase, it seems that the presence of other phenolic components may potentiate the inhibition. This could occur by stabilizing the ETs/PACs (thus, maintaining their inhibition), by more subtly affecting the propensity of amylase to interact with the tannin components or directly inhibiting amylase.

Flavonols and flavone aglycones have been found to inhibit α -amylase *in vitro*,³⁶ with IC_{50} values at micromolar levels (equivalent to approximately $6 \mu\text{g}/\text{mL}$ for quercetin). Modeling studies suggested that these components act by binding at the active site,³⁶ which could be synergistic with inhibition by the protein-binding effect of PACs or ETs. Flavonol aglycones are rare in berries,² but glycosylated flavonols are common³⁷ and were present in the raspberry (Figure 3) and rowanberry extracts²² used in this study. However, an initial report suggested that glycosylated flavonols were considerably less effective than their aglycones;³⁸ therefore, their ability to inhibit in these berry extracts may be limited. The potential additive or synergistic effect of phenolic components, such as flavonols or anthocyanins, needs to be examined. In the first instance, studies that examine the effect of recombination of fractions (such as tannin-rich, flavonol-enriched, and anthocyanin-rich fractions) on amylase inhibition are warranted.

The co-addition of acarbose and rowanberry PACs also suggested that these components had an additive or synergistic effect. The potent inhibition achieved by the addition of PACs and acarbose at 75:25% of their IC_{50} values suggests that PACs could be used to reduce the dose of acarbose required for glycaemic control. Also, these results suggest that acarbose and

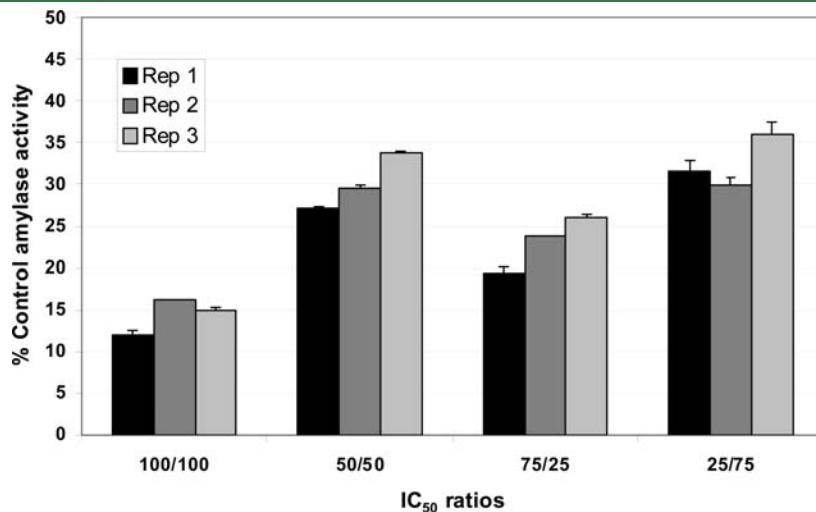


Figure 7. Effect of co-incubation with acarbose and rowanberry PACs. The percent control activity was assessed for each set of assays. The values shown are averages of triplicate assays \pm standard error. Acarbose and rowanberry PACs were added at various defined ratios of their IC_{50} values: e.g., 100/100 = each at 100% of their IC_{50} values, and 75/25 = 75% IC_{50} value of rowanberry and 25% IC_{50} value of acarbose. Three replicate assays are shown, run on different days.

PACs operate by binding at different sites, which prevents competition and potentiates overall inhibition. Acarbose acts in a mixed non-competitive manner and binds to a site other than the active site.²⁶

Acarbose is an effective treatment for modulating glycaemic control in subjects with type-2 diabetes mellitus,¹⁵ but it has side-effects mainly concerning gastrointestinal discomfort. Co-application with PACs may have the potential to reduce the dose of acarbose required and reduce side-effects. It is intriguing that cyanidin-3-glucoside at low levels has also been found to potentiate amylase inhibition *in vitro* by acarbose.³⁹ Indeed, studies have suggested that cyanidin-3-glucoside binds to α -amylase and may alter its tertiary structure.⁴⁰

Studies⁴¹ indicate that berry polyphenols could be available in the small intestine at concentrations that cause inhibition of amylase *in vitro*. The components identified as important inhibitors in this study (PACs in rowanberry and ETs in raspberry) have been reported to undergo partial breakdown under real or simulated gastrointestinal conditions,^{42–44} but initial studies suggest that berry extracts subjected to *in vitro* digestion also retain amylase inhibitory activity to a large degree (results not shown).

Animal model studies have shown that polyphenol-rich berry extracts can ameliorate hyperglycaemia,⁴⁵ and there are indications that berry components may be effective in humans.^{46,47} The limited studies on berry components build on a wealth of evidence that other polyphenol-rich extracts can influence glycaemic control in animal models, often as a result of inhibition of starch digestion (such as grape pomace polyphenols⁴⁸). In summary, these *in vitro* results indicate that berry polyphenols inhibit α -amylase at levels easily achieved in the GIT through normal dietary intake of fruits or juices. Considering that similar berry extracts have been shown to inhibit α -glucosidase *in vitro*,⁴⁹ there is obvious potential for synergy in the inhibition of starch digestion in the GIT. Finding a balance between α -amylase and α -glucosidase inhibition may be important to limit gastrointestinal side-effects associated with undigested starch reaching the colon. However, there are a number of foreseeable problems. For example, the presence of other dietary components, such as proteins, polysaccharides,³⁴ and bile secretions, may protect against inhibition by polyphenols. Ultimately, further human studies will be required to discover if these effects can be transferred to the *In Vivo* situation.

ASSOCIATED CONTENT

Supporting Information. Illustration of the rowanberry fractionation (Figure S1), data on the effect of changing the order of the addition of amylase assay components (Figure S2), and identification of phenolic components in red and yellow raspberry extracts (Table S1). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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