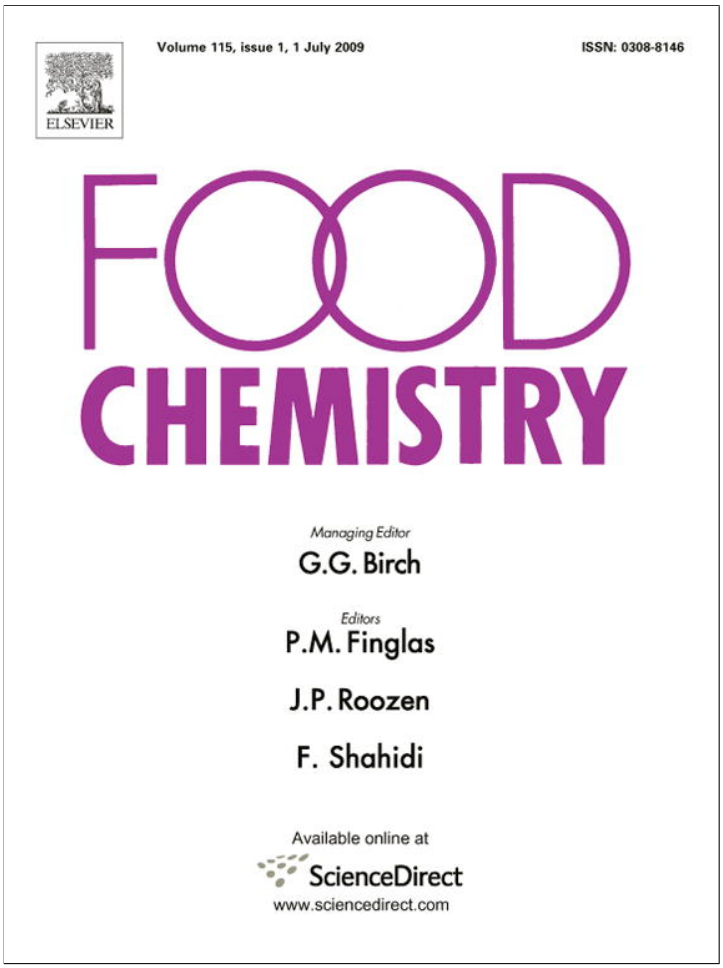

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Berry polyphenols inhibit pancreatic lipase activity *in vitro*

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ABSTRACT

Polyphenol-rich extracts from a range of berries were tested for their ability to inhibit pancreatic lipase activity *in vitro*. Blackcurrant and rowan extracts had no effect, blueberry caused slight inhibition, whilst lingonberry, Arctic bramble, cloudberry, strawberry and raspberry were considerably more effective. Inhibition by the cloudberry extract showed a saturation effect with an apparent EC_{50} of around 5 μg phenols/ml.

The inhibitory components from cloudberry were retained in a tannin-rich fraction prepared by sorption to Sephadex LH-20. Comparison of the polyphenol composition of the active and inactive fractions using liquid chromatography–mass spectrometry (LC–MS) strongly suggested that the active components were ellagitannins. Similarly prepared fractions from raspberry and strawberry were also effective inhibitors. Direct infusion mass spectra (DIMS) of the raspberry tannin and cloudberry tannin fractions were very similar, with minor differences in the abundance of certain ellagitannin components, whereas the strawberry tannin fraction was enriched in a mixture of ellagitannin and proanthocyanidin components. The effective inhibition of lipase by the lingonberry extract may have been due to proanthocyandin components. The nature, mechanism and possible physiological relevance of lipase inhibition by berry components are discussed.

1. Introduction

According to a recent World Health Organisation report, there is an epidemic of obesity threatening the health of Western nations (Anon, 2003). In the United Kingdom, 1 in 4 adults can be termed obese (Anon, 2007) with all projections suggesting an increasing trend over the next decades (Zaninotto et al., 2006). As well as being a debilitating condition in its own right, obesity is a key contributing risk factor for cardiovascular disease, certain cancers, diabetes and the combination of risk factors known as Metabolic Syndrome (Lean, Lara & O'Hill, 2006). An imbalance between calorie intake and metabolic expenditure is a central factor in many cases of obesity and reductions in intake of energy dense fats may be useful to reduce weight (Mulvihill & Quigley, 2003). The suppression of energy intake by inhibiting the action of pancreatic lipase, which splits triglycerides into absorbable glycerol and fatty acids (Lowe, 1997), by drugs such as Orlistat (Sjöström et al., 1998), has been employed to treat obesity.

There have been reports that naturally-occurring polyphenols can inhibit pancreatic lipase and thereby influence fat digestion and affect energy intake (McDougall & Stewart, 2005). Many studies have focused on polyphenols from teas and herbal sources (Birari & Bhutani, 2007) but there is also evidence that polyphenols from fruit sources can also inhibit this enzyme (e.g., Moreno, Ilic, Poulev, Brasaemleet, Fried, & Raskin, 2003). This paper screens a range of polyphenol-rich berry extracts and their sub-fractions for effects on pancreatic lipase that could be relevant to fat digestion, energy intake and obesity. By comparing the phytochemical diversity of the berry extracts with their anti-lipase effectiveness, key structural components may be identified.

2. Materials and methods

2.1. Extraction of berries

Blackcurrants (*Ribes nigrum* L. variety 8982-6) were obtained from Bradenham Hall, Norfolk, UK and blueberries (*Vaccinium corymbosum* variety, Berkeley) were grown at SCRI. Cloudberries (*Rubus chamaemorus*), Arctic bramble (*Rubus stellatus* × *R. arcticus*), lingonberries (*Vaccinium vitis-idaea*) and rowan berries (*Sorbus aucuparia* variety Sahharnaja) were obtained from Dr. Harri Kokko, University of Kuopio. Strawberries (*Fragaria ananassa* variety Elsanta) and raspberries (*Rubus idaeus* variety Glen Ample) were obtained from local farmers. All fruit were picked at full ripeness and frozen within a day of picking and, where required, then transported frozen to SCRI.

These were extracted by the protocol outlined previously (Ross, McDougall, & Stewart, 2007). Briefly, the berries were

Abbreviations: DIMS, direct infusion mass spectra; GAE, gallic acid equivalents; SPE, solid phase extraction.

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homogenised in an equal volume to weight of 0.2% (v/v) formic acid in water using a Waring blender (5 times for 15 s on full power). The extract was filtered through tripled muslin then centrifuged at 4000 rpm for 10 min at 4 °C to remove suspended polysaccharides and pulp.

The extracts were applied to C_{18} solid phase extraction (SPE) units (Strata C18-E, GIGA units, 10 g capacity; Phenomenex Ltd., Macclesfield, UK) pre-washed in 0.2% (v/v) formic acid in acetonitrile 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 then with 2 volumes of ultra-pure water. The polyphenol-enriched bound extracts eluted with acetonitrile. The C_{18}-bound extracts were evaporated to dryness in a SpeedVac (Thermo Scientific, Waltham, MA).

A second cloudberry extract was prepared by extracting 400 g cloudberry puree in 400 ml of 0.2% (v/v) formic acid, as above. The cloudberry puree was produced in Kuopio using a Robot Coupe C200 machine which separates the seeds from the berries. The bulk of this extract was applied to C_{18} SPE units, as described above.

A portion of the new cloudberry extract was freeze dried and redissolved in 50% (v/v) ethanol and centrifuged to remove insoluble material (as above) then applied to chromatography on Sephadex LH-20, using the method outlined in the Tannins Handbook (at www.users.muohio.edu/hagermae/tannin.pdf). Briefly, Sephadex LH-20 was swollen in 50% (v/v) ethanol/water, poured into a glass column, washed with 50% (v/v) acetone/water before being equilibrated with three volumes of 50% ethanol.

The run-through material plus a column volume of 50% ethanol was collected as the unbound fraction. The wash sample was obtained when the column was washed with three column volumes of 50% ethanol. The bound fraction was eluted with three volumes of 50% acetone. The unbound and wash samples were rotary evaporated to remove ethanol and applied to C_{18} SPE units, as described above. This ensured that they were enriched in polyphenols and comparable to the original extract.

Strawberry and raspberry extracts were fractionated on Sephadex LH-20 in essentially the same way, to prepare bound tannin-rich samples.

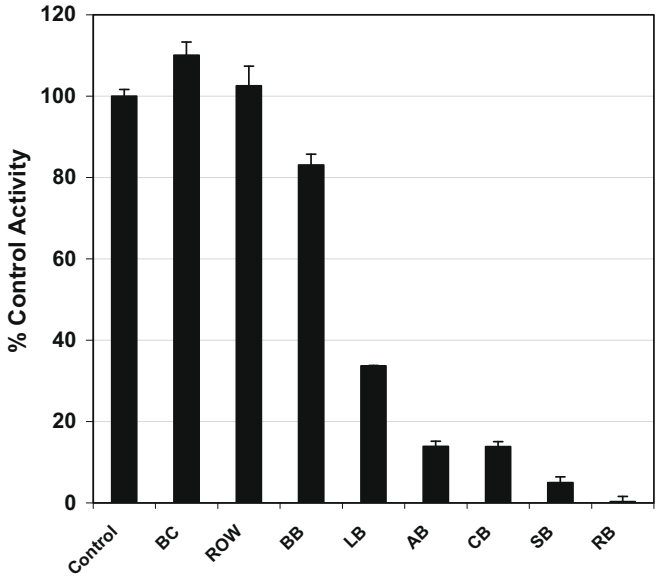

Fig. 1. Screen for inhibition of lipase inhibition by berry extracts. All extracts were tested at 50 μg phenol content (GAE). BC = blackcurrant; ROW = rowan; BB = blueberry; LB = lingonberry; AB = Arctic bramble; CB = cloudberry; SB = strawberry; RB = raspberry. A representative experiment is shown and values are means of triplicate assays ± standard error.

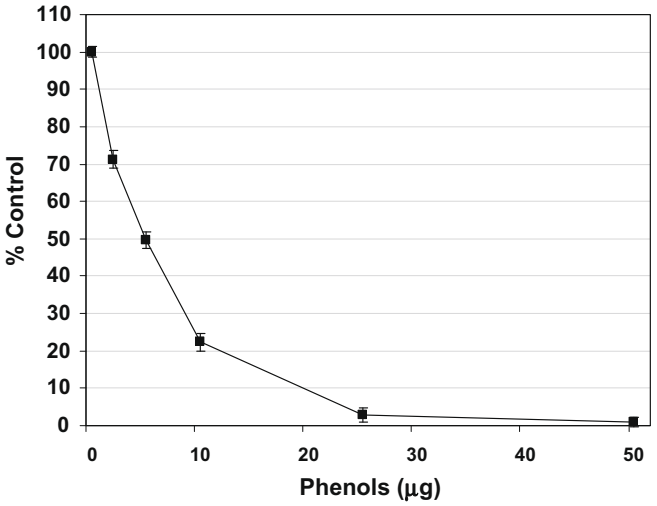

Fig. 2. Inhibition of lipase activity by cloudberry extract. Values are means of triplicate assays ± standard error.

Phenol content was measured using a modified Folin–Ciocalteu method (Deighton, Brennan, Finn, & Davies, 2000) and quantified as gallic acid equivalents (GAE). All samples were dried in aliquots of suitable phenol content in a Speed-Vac prior to assay.

2.2. Hexane extraction

Cloudberry extract containing 500 μg phenols (GAE) was dissolved in 500 μl distilled water; then 1 ml *n*-hexane was added and shaken for 10 min at 100 rpm at room temperature. After centrifugation for 2 mins at 16,000 rpm in a microcentrifuge, the upper (hexane) phase was removed into a new tube. The procedure was repeated and the hexane phases combined. The water phase and hexane phase were dried using a SpeedVac. The water phase was resuspended in 500 μl of distilled water and checked for phenol content. The hexane phase was dried and stored in the freezer. The water phase and hexane phase were tested for enzyme inhibition at different concentrations.

2.3. Lipase assay

This assay was adapted from previous reports (Gilham & Lehner, 2005; Lin, Chiou, Yeh, & Tsai, 1996). Lipase from porcine pancreas Type II (Sigma product L3126) was dissolved in ultra-pure water at 10 mg/ml; then the supernatant was used after centrifugation at 16,000 rpm for 5 min. The assay buffer was 100 mM Tris buffer (pH 8.2) and *p*-nitrophenyl laurate (pNP laurate) was used as the substrate. The substrate stock was 0.08% w/v pNP laurate dissolved in 5 mM sodium acetate (pH 5.0) containing 1% Triton X-100 and was heated in boiling water for 1 min to aid dissolution, mixed well, then cooled to room temperature.

Table 1
Effect of fractionation of cloudberry extracts using lipophilic Sephadex on lipase inhibition.

	% Control lipase		% Control pancreatin
Samples	25 μg	50 μg	50 μg
Original extract	2.8 ± 0.4	0.1 ± 0.4	22.9 ± 2.1
Puree extract*	14.3 ± 1.2	1.7 ± 0.5	54.1 ± 4.3
LH-20 unbound*	95.6 ± 1.8	95.0 ± 1.3	98.0 ± 1.6
LH-20 bound*	16.5 ± 0.9	7.1 ± 0.8	43.8 ± 1.3

* The LH-20 fractionation was carried out on the cloudberry puree extract.

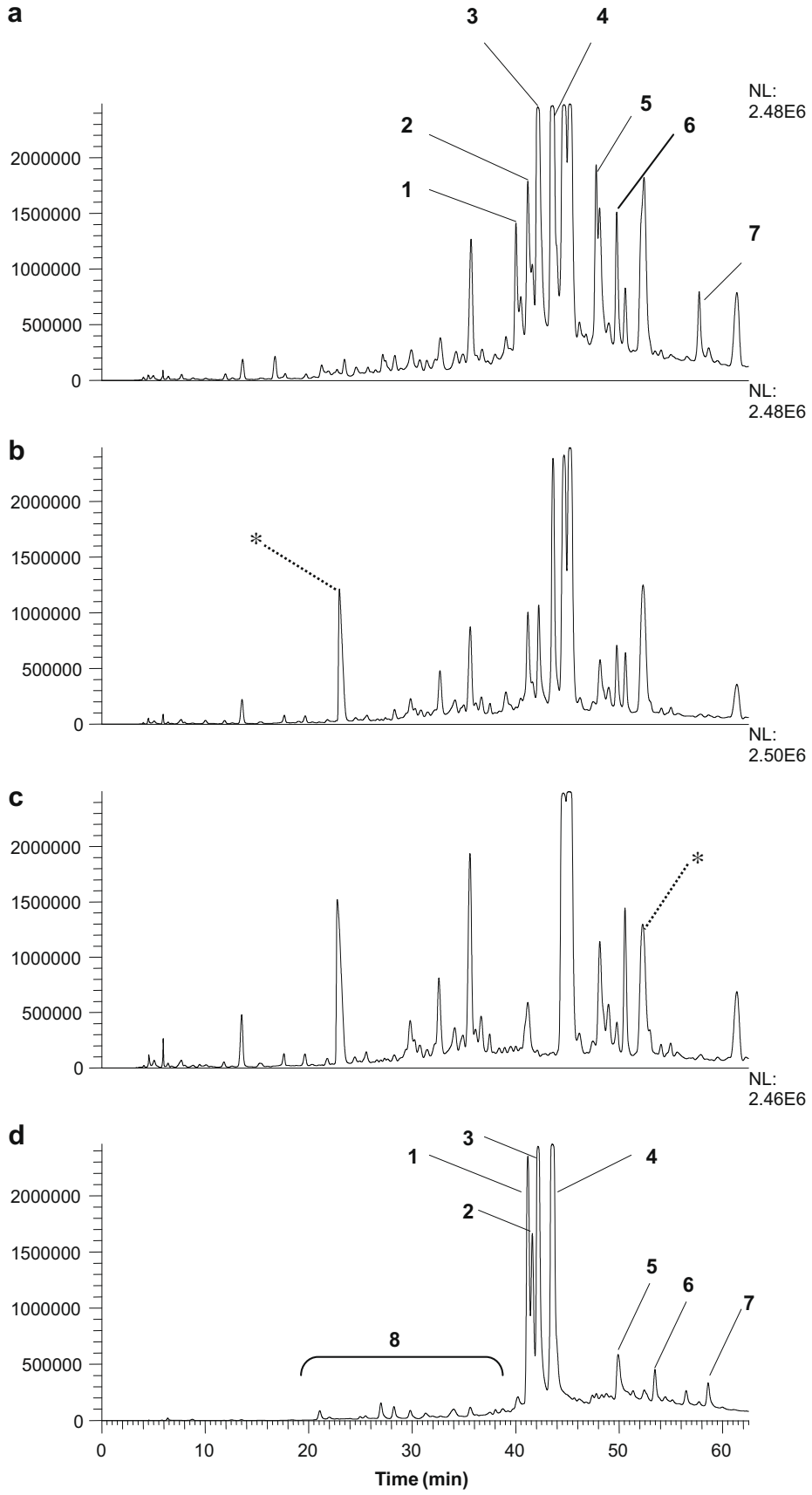

Fig. 3. LC–MS traces of cloudberry extracts. Each trace is from 20 μg GAE. (a) Is the original extract; (b) is the cloudberry puree extract; (c) is the LH-20 unbound fraction and (d) is the LH-20 bound fraction. Peaks annotated with asterisks are examples of peaks that are unlikely to contribute to lipase inhibition.

Table 2
Putative identities of cloudberry LC–MS peaks.

Peak	PDA	*m/z* [M–H]	MS^2	Putative identity
1	275	**1567.1**, 934.2, 783.3, 633.1, 301.2	1264.9, 1234.9, 1102.8, 932.9, 897.0	Sanguiin H6 isomer
2	275	**1567.1**, 934.2, 783.3, 633.1, 301.2	1264.9, 1234.9, 1102.8, 932.9, 897.0	Sanguiin H6 isomer
3	275	**1401.3,** 1868.8, 1566.9, 934.2, 633.1, 301.2	1866.9, 1566.9, 1250.2, 1234.9, 897.1	Lambertinian C
4	275	**1868.9,** 1566.9, 1234.9, **934.2**, 633.2, 301.2	1566.8, 1234.9, 933.0	Sanguiin H10
5	370	**301.2**, 275.1	275.1	Ellagic acid
6	275	**1085.2,** 633.2, 451.2, 301.2	633.2, 451.2	Unknown ellagitannin
7	275	1868.9, 1234.9, 935.2, 633.2, **301.2,** 275.1	Multiple	Sanguiin H10 isomer
8	260–290	Multiple	Multiple	Ellagic acid conjugates and ellagitannins

The peak annotations refer to LC–MS data on Figure 3. Figures in bold are the main *m/z* signals and those underlined are the major MS^2 products.

The control assay contained 400 μl assay buffer, 450 μl substrate solution and 150 μl lipase. Berry extracts were dissolved in ultra-pure water and added in 50 μl total volume. The buffer, enzyme and berry extracts were added and then substrate was added to start the reaction. The samples were incubated at 37 °C for 2 h. Then samples were centrifuged at 16,000 rpm for 2.5 min and read at 400 nm in a UV spectrophotometer. All samples were assayed in triplicate and an inhibitor blank was prepared for each sample.

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

Berry samples containing 20 μg phenols (gallic acid equivalents; GAE) were analysed on an LCQ-Deca system, comprising Surveyor autosampler, pump and photodiode array detector (PDAD) and a ThermoFinnigan ion-trap mass spectrometer. The PDAD scanned discrete channels at 280 nm, 365 nm and 520 nm. The samples were applied to a C_{18} column (Synergi Hydro C_{18} with polar endcapping, 4.6 mm × 150 mm, Phenomenex Ltd.) and eluted using a gradient of 5% acetonitrile (0.5% formic acid) to 30% acetonitrile (0.5% formic acid) over 60 min at a rate of 400 μl/min. The LCQ-Deca LC–MS was fitted with an ESI (electrospray ionisation) interface and analysed the samples 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 using collision energies (source voltage) of 45%. The capillary temp was set at 250 °C, with sheath gas at 60 psi and auxiliary gas at 15 psi.

2.5. Direct infusion mass spectrometry (DIMS)

Direct infusion mass spectrometry (DIMS) analysis of samples was carried out on an LCQ-Deca (ThermoFinnigan) controlled by the Xcalibur software (Version 1.4, ThermoFinnigan). The mode of ionisation was ESI in negative ion mode, scanning from *m/z* 80–2000. The sample was injected into the LC–MS mobile phase (200 μl/min of 50% acetonitrile containing 0.1% (v/v) formic acid) and then directly into the ESI source. Data was acquired for 2 mins and each sample was followed by three blank injections (solvent only) to ensure no carry-over between samples. The following parameters were used: capillary temperature: 275 °C, capillary voltage: 20 V, spray voltage: 5 kV, tube lens: −5 V, sheath gas: 70 arbitrary units and auxiliary gas: 15 arbitrary units. The autosampler (Surveyor AS, ThermoFinnigan) tray temperature control was set at 4 °C. Triplicate injections were made in a randomised fashion and the peak heights of the relevant spectral peaks averaged. The peaks heights in blank spectra were subtracted.

3. Results

Polyphenol-rich extracts of berries and other fruits at 50 μg/ml GAE phenols were tested for their ability to inhibit pancreatic lipase *in vitro* (Fig. 1). The extracts had different effects; blackcurrant and rowan had no effect, the blueberry extract caused slight but significant inhibition whilst lingonberry, Arctic bramble, cloudberry, strawberry and raspberry were particularly effective.

The inhibition by the cloudberry extract was saturatable with an EC_{50} (concentration for half maximal activity) of around 5 μg/ml phenols (Fig. 2). The cloudberry extract was extracted with hexane to remove lipid-like components and this sample was as effective as the original sample (results not shown). This strongly suggests that the inhibition was not due to lipid derivatives in the cloudberry extract.

The original cloudberry sample was more effective than the extract prepared from cloudberry puree and this difference in effectiveness was also apparent in the inhibition of the lipase activity in the crude pancreatin mixture (Table 1). The inhibition of lipase in the crude pancreatin mixture may be closer to physiological conditions in the small intestine where interactions of the polyphenols with other proteins, mucins and cell surfaces may protect lipase activity. The difference in effectiveness between the two cloudberry extracts is probably due to differences in the polyphenolic composition arising from the presence of seeds in the original extract.

Indeed, the original cloudberry extract gave a different polyphenol composition on LC–MS trace than the cloudberry puree sample (compare traces Fig. 3a and b). It is apparent that the original extract contained comparably higher amounts of peaks 1–3 and 5–7 and smaller amounts of other polyphenol components (Table

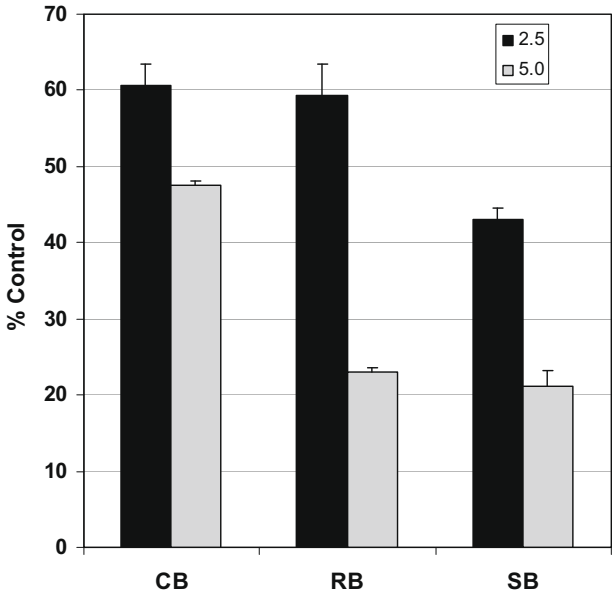

Fig. 4. Berry tannin fractions show differential inhibition of lipase CB = cloudberry; RB = raspberry and SB = strawberry. Values are means of triplicate assays ± standard error.

2). The abundance of these ellagitannin derivatives may explain the enhanced inhibitory effectiveness of the original cloudberry extract over the puree extract. It has been previously noted that ellagitannin components are enriched in seeds (e.g., Hager, Howard, Liyanage, Lay, & Prior, 2008).

The fractionation on Sephadex LH-20 split the polyphenolic components in the cloudberry puree sample (Fig. 3b) between the unbound (Fig. 3c) and bound samples (Fig. 3d). As the lipase inhibitory activity remained in the bound sample (Table 1), certain peaks which fractionate into the unbound sample can be discounted (see Fig. 3, marked with asterisks). The bound sample is composed of peaks that can be identified as ellagitannins and ellagic acid by comparison with previous work (Hager et al., 2008; McDougall, Martinussen, & Stewart, 2008; Mullen, Yokota, Lean, & Crozier, 2003; Ross et al., 2007) (Table 2).

Lipase activity was also effectively inhibited by tannin-rich extracts of raspberry and strawberry (Fig. 4), prepared by chromatography on Sephadex LH-20. The raspberry and strawberry tannin extracts were more effective than cloudberry, which follows the pattern of the whole berry extracts (Fig. 1). The diversity of the polyphenol composition of these tannin-rich extracts can be displayed by their direct infusion mass spectra (DIMS) (Fig. 5). The raspberry and cloudberry spectra contain similar signals (*m/z* 1868.9, 1566.9, 935.1, 783.1 and 301.2), which can be assigned to ellagitannin structures. They differ mainly in the greater abundance of the *m/z* signal at 1401.2 in cloudberry, which can be assigned to the presence of Lambertinian C (Hager et al., 2008; McDougall, Ross, Ikeji, & Stewart, 2008; Mullen et al., 2003; Ross et al., 2007). The strawberry tannin sample showed signals characteristic of ellagitannin structures previously identified in strawberry (i.e., *m/z* 1868.9, 1566.9, 935.1, 783.1 and 301.2) but also had signals at *m/z* 1729.2, 1441.0, 1153.1, 865.1 and 577.1 (in bold), which are characteristic of procyanidin proanthocyanidins (Gu et al., 2003). In addition, there are signals at *m/z* 1425.0, 1409.0, 1137.0, 1121.1 and 849.1 (noted in gray), which are characteristic of propelargonidin proanthocyanidins also previously identified in strawberry (Gu et al., 2003).

4. Discussion

Polyphenol-rich extracts from certain berries are effective inhibitors of pancreatic lipase *in vitro*. The most effective berry types were three members of the *Rubus* family (raspberry, Arctic bramble and cloudberry) and strawberry. Published polyphenolic compositions of these berry types (Hakkinen, Heinonen, Karenlampi, Mykkanen, Ruuskanen, & Törrönen, 1999; Kahkonen, Hopia, & Heinonen, 2001; Maatta-Riihinen, Kamal-Eldin, Matiila, Gonzalez-Paramas, & Törrönen, 2004; Machiex & Billot, 1990) suggest that ellagitannins could be important for lipase inhibition. In addition, the effective inhibition caused by raspberry and cloudberry extracts suggests that anthocyanins, which are present in only small amounts in cloudberry (Maatta-Riihinen et al., 2004), are not necessary for lipase inhibition. Nevertheless, it is possible that synergistic interactions between polyphenols occur, even in terms of protecting the stability of the active components.

The importance of ellagitannins was borne out by the retrieval of lipase inhibitory activity in the cloudberry tannin-rich bound fraction after fractionation on Sephadex LH-20 (Table 1 and Fig. 3) and the effectiveness of raspberry and strawberry tannin-rich fractions (Fig. 4). The greater effectiveness of the raspberry tannin extract over the cloudberry tannin extract (Fig. 5) suggests that Lambertinian C, which is more abundant in the cloudberry ex-

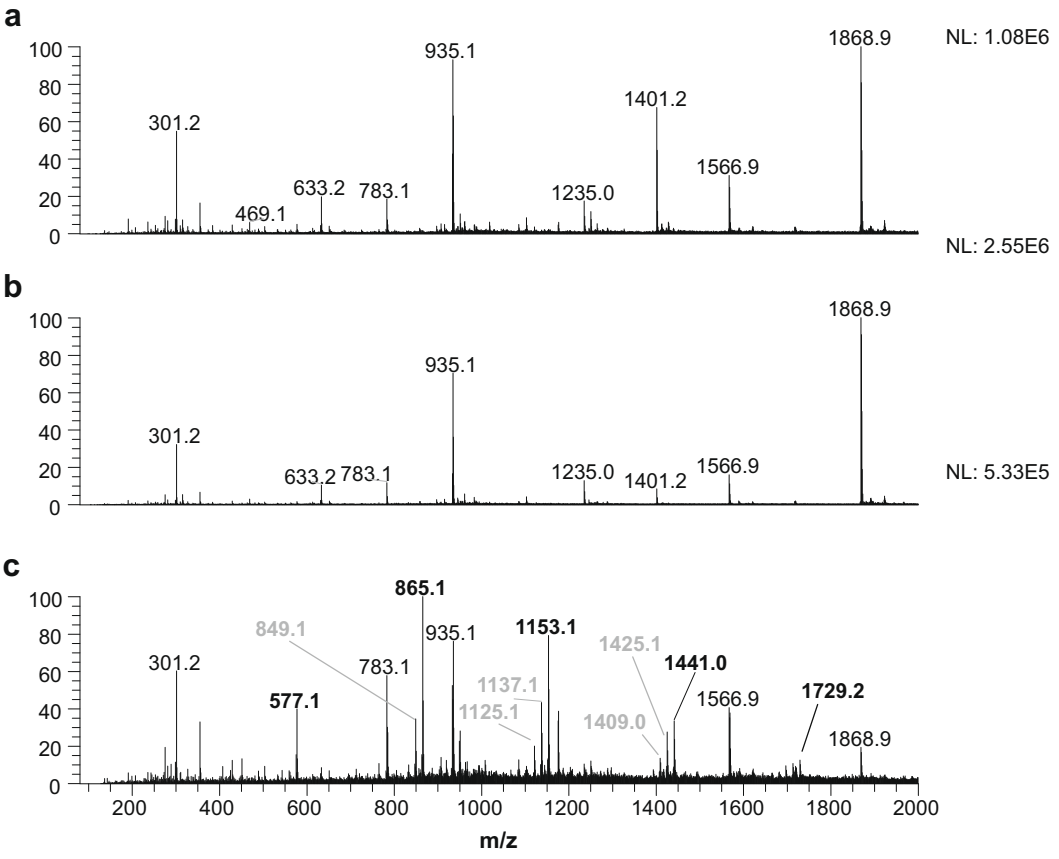

Fig. 5. Direct infusion mass spectra (DIMS) of berry tannin fractions (a) is the cloudberry tannin fraction; (b) is the raspberry tannin fraction and (c) is the strawberry tannin fraction. In each case, 2 μg phenols (GAE) was injected. Annotated masses are discussed in the text.

tract, may not be a particularly effective lipase inhibitor. If we assume that the protein-binding affinity of the ellagitannins is important to lipase inhibition (which is suggested by the lower inhibition of lipase in the crude pancreatin sample), it may be that different ellagitannin structures have different lipase binding affinities. However, although differences in protein-binding affinity have been noted for certain purified ellagitannins (e.g., Deaville, Green, Mueller-Harvey, Willoughby, & Frazier, 2007), further work on berry ellagitannins is required to confirm this possibility.

The inhibition caused by the strawberry extract and the strawberry tannin fraction may have been influenced by proanthocyanidins, which have previously been reported as effective lipase inhibitors in grape seed extracts (Moreno et al., 2003). In addition, oligomeric proanthocyanidins from apple have been shown to be the main active components responsible for inhibition of pancreatic lipase *in vitro* and for the prevention of triglyceride absorption in humans and in mice models (Sugiyama et al., 2007). Therefore, it also seems likely that proanthocyanidin components present in lingonberry extracts (McDougall et al., 2008) are responsible for their lipase inhibition.

Polyphenols from red wine can delay the absorption of dietary fat in humans (Pal, Naissides, & Mamo, 2004) and reduce obesity in rats (Vadillo et al., 2006). Similar effects on obesity have been identified for tea polyphenols (Kurihara et al., 2006; Wolfram, Wang, & Thielecke, 2006) and they have also been reported to inhibit pancreatic lipase *in vitro* (Kurihara et al., 2006; Juhel et al., 2000; Nakai et al., 2005). Other reports have highlighted the possibility that dietary anthocyanins could regulate adipocyte function and influence obesity (Tsuda, 2008; Tsuda, Horio, Uchida, Aoki, & Osawa, 2003) or reduce weight gain and obesity in mice models Prior et al., 2008; Jayaprakasam, Olson, Schutzki, Tai, & Nair, 2006).

Model *in vitro* digestion studies (McDougall, Dobson, Fyffe, Shpiro, & Stewart, 2007) and studies with colostomy patients (Karle et al., 2006) have confirmed that berry polyphenols would be available in the small intestine at concentrations that inhibit pancreatic lipase *in vitro*. However, inhibition of lipase is only one of many mechanisms whereby polyphenols could influence obesity (Hsu & Yen, 2007) and it is possible that inhibition of lipase would be overcome by compensatory increases in lipase secretion, as seen in studies on condensed tannins in rats (Griffiths, 1986). Indeed, certain studies have suggested that high polyphenol intake does not increase faecal lipid excretion, which would be expected if lipase was inhibited (Tsuda, 2008). Further work is required to confirm that berry components can inhibit lipase *in vivo*, prevent fat intake and do so at levels achievable from normal diets. This is particularly relevant as whole berries did not prevent fat gain in model mice when isolated berry polyphenols were effective (Prior et al., 2008).

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References

Anon (2003). *Obesity and Overweight – Facts*. World Health Organisation Report. Available at http://www.who.int/dietphysicalactivity/media/en/gsfs_obesity.pdf.

Anon (2007). *Obesity in Scotland: An epidemiology briefing*. Scottish Public Health Observatory Publication. Available at http://www.scotpho.org.uk/home/Publications/scotphoreports/pub_obesityinscotland.asp.

Birari, R. B., & Bhutani, K. K. (2007). Pancreatic lipase inhibitors from natural sources: Unexplored potential. *Drug Discovery Today, 12*, 879–889.

Deaville, E. R., Green, R. J., Mueller-Harvey, I., Willoughby, I., & Frazier, R. A. (2007). Hydrolyzable tannin structures influence relative globular and random coil protein binding strengths. *Journal of Agricultural and Food Chemistry, 55*, 4554–4561.

Deighton, N., Brennan, R., Finn, C., & Davies, H. V. (2000). Antioxidant properties of domesticated and wild Rubus species. *Journal of the Science of Food and Agriculture, 80*, 1307–1313.

Gilham, D., & Lehner, R. (2005). Techniques to measure lipase and esterase activity in vitro. *Methods, 36*, 139–147.

Griffiths, D. W. (1986). The inhibition of digestive enzymes by polyphenolic compounds. *Advances in Experimental Medicine and Biology, 199*, 509–516.

Gu, L., Kelm, M. A., Hammerstone, J. F., Beecher, G., Holden, J., Haytowitz, D., & Prior, R. L. (2003). Screening of foods containing proanthocyanidins and their structural characterization using LC–MS and thiolytic degradation. *Journal of Agricultural and Food Chemistry, 51*, 7513–7521.

Hager, T. J., Howard, L. R., Liyanage, R., Lay, J. O., & Prior, R. L. (2008). Ellagitannin composition of blackberry as determined by HPLC–ESI–MS and MALDI–TOF–MS. *Journal of Agricultural and Food Chemistry, 56*, 661–669.

Hakkinen, S., Heinonen, M., Karenlampi, S., Mykkanen, H., Ruuskanen, R., & Törrönen, R. (1999). Screening of selected flavonoids and phenolic acids in 19 berries. *Food Research International, 32*, 345–353.

Hsu, C-L., & Yen, G-C. (2007). Phenolic compounds: Evidence for inhibitory effects against obesity and their underlying molecular signaling mechanisms. *Molecular Nutrition and Food Research, 52*, 53–61.

Jayaprakasam, B., Olson, L. K., Schutzki, R. E., Tai, M.-H., & Nair, M. G. (2006). Amelioration of obesity and glucose intolerance in high-fat-fed C57BL/6 mice by anthocyanins and ursolic acid in cornelian cherry (*Cornus mas*). *Journal of Agricultural and Food Chemistry, 54*, 243–248.

Juhel, C., Armand, M., Pafumi, Y., Rosier, C., Vandermander, J., & Lairon, D. (2000). Green tea extract (AR25®) inhibits of lipolysis of triglycerides in gastric and duodenal medium in vitro. *Journal of Nutrition and Biochemistry, 11*, 45–51.

Kahkonen, M., Hopia, A., & Heinonen, M. (2001). Berry phenolics and their antioxidant activity. *Journal of Agricultural and Food Chemistry, 49*, 4076–4082.

Karle, K., Kraus, M., Scheppach, W., Ackermann, M., Ridder, F., & Richling, E. (2006). Studies on apple and blueberry fruits constituents: Do the polyphenols reach the colon after ingestion? *Molecular Nutrition and Food Research, 50*, 418–423.

Kurihara, H., Shibata, H., Fukui, Y., Kiso, Y., Xu, J.-K., Yao, X.-S., et al. (2006). Evaluation of the hypolipemic property of *Camellia sinensis var. ptilophylla* on postprandial hypertriglyceridemia. *Journal of Agricultural and Food Chemistry, 54*, 4977–4981.

Lean, M., Lara, J., & O'Hill, J. (2006). ABC of obesity: Strategies for preventing obesity. *British Medical Journal, 333*, 959–962.

Lin, S-F., Chiou, C-M., Yeh, C-M., & Tsai, Y-C. (1996). Purification and Partial Characterization of an alkaline lipase from *Pseudomonas pseudoalcaligenes* F-111. *Applied and Environmental Microbiology, 62*, 1093–1095.

Lowe, M. E. (1997). Structure and function of pancreatic lipase and colipase. *Annals Review Nutrition, 17*, 141–158.

Maatta-Riihinen, K. R., Kamal-Eldin, A., Matiila, P. H., Gonzalez-Paramas, A. M., & Törrönen, R. (2004). Distribution and contents of phenolic compounds in eighteen Scandinavian berry species. *Journal of Agricultural and Food Chemistry, 52*, 4477–4486.

Machiex, J. F. A., & Billot, J. (1990). *Fruit phenolics*. Florida, USA: CRC Press.

McDougall, G. J., & Stewart, D. (2005). The inhibitory effects of berry polyphenols on digestive enzymes. *Biofactors, 23*, 189–195.

McDougall, G. J., Dobson, P., Fyffe, S., Shpiro, F., & Stewart, D. (2007). Assessing bioavailability of soft fruit polyphenols in vitro. *Acta Horticultural, 744*, 135–148.

McDougall, G. J., Ross, H. A., Ikeji, M., & Stewart, D. (2008). Berry extracts exert different anti-proliferative effects against cervical and colon cancer cells grown in vitro. *Journal of Agricultural and Food Chemistry, 56*, 3016–3023.

McDougall, G., Martinussen, I., & Stewart, D. (2008). Towards fruitful metabolomics: High throughput analyses of polyphenol composition in berries using direct infusion mass spectrometry. *Journal of Chromatography B*, 362–369.

Moreno, D. A., Ilic, N., Poulev, A., Brasaemleet, D., Fried, S. K., & Raskin, I. (2003). Inhibitory effects of grape seed extract on lipases. *Nutrition, 19*, 876–879.

Mullen, W., Yokota, T., Lean, M. E. J., & Crozier, A. (2003). Analysis of ellagitannins and conjugates of ellagic acid and quercetin in raspberry fruits by LC–MS_n. *Phytochemistry, 64*, 617–624.

Mulvihill, C., & Quigley, R. (2003). *The management of obesity and overweight: An analysis of reviews of diet, physical activity and behavioural approaches*. London: Health Development Agency.

Nakai, M., Fukui, Y., Asami, S., Toyoda-Ono, Y., Iwashita, T., Shibata, H., et al. (2005). Inhibitory effects of oolong tea polyphenols on pancreatic lipase in vitro. *Journal of Agricultural and Food Chemistry, 53*, 4593–4598.

Pal, S., Naissides, M., & Mamo, J. (2004). Polyphenolics and fat absorption. *International Journal of Obesity, 28*, 324–326.

Prior, R. L., Wu, X., Gu, L., Hager, T. J., Hager, A., & Howard, L. R. (2008). Whole berries versus berry anthocyanins: Interactions with dietary fat levels in the C57BL/6J mouse model of obesity. *Journal of Agricultural and Food Chemistry, 56*, 647–653.

Ross, H. A., McDougall, G. J., & Stewart, D. (2007). Antiproliferative activity is predominantly associated with ellagitannins in raspberry extracts. *Phytochemistry, 68*, 218–228.

Sjöström, L., Rissanen, A., Andersen, T., Boldrin, M., et al. (1998). Randomised placebo-controlled trial of Orlistat for weight loss and prevention of weight regain in obese patients. *Lancet, 352*, 167–172.

Sugiyama, H., Akazome, Y., Shoji, T., Yamaguchi, A., Yasue, M., Kanda, T., et al. (2007). Oligomeric procyanidins in apple polyphenol are main active components for inhibition of pancreatic lipase and triglyceride absorption. *Journal of Agricultural and Food Chemistry, 55*, 4604–4609.

Tsuda, T., Horio, F., Uchida, K., Aoki, H., & Osawa, T. (2003). Dietary cyanidin-3-*O*-β-glucoside rich purple corn color prevents obesity and ameliorates hyperglycemia in mice. *Journal of Nutrition, 133*, 2125–2130.

Tsuda, T. (2008). Regulation of adipocyte function by anthocyanins: Possibility of preventing the metabolic syndrome. *Journal of Agricultural and Food Chemistry, 56*, 642–646.

Vadillo, M., Ardévol, A., Fernández-Larrea, J., Pujadas, G., Bladé, C., & Salvadó, M. J. (2006). Moderate red wine consumption partially prevents weight gain in rats fed a hyperlipidic diet. *Journal of Nutritional Biochemistry, 17*, 139–142.

Wolfram, S., Wang, Y., & Thielecke, F. (2006). Anti-obesity effects of green tea: From bedside to bench. *Molecular Nutrition and Food Research, 50*, 176–187.

Zaninotto, P., Wardle, H., Stamatakis, E., Mindell, J., & Head, J. (2006). Forecasting Obesity to 2010. National Centre for Social Research and University College of London; London. Available at http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsStatistics/DH_4138630.