Food Research International 43 (2010) 1537–1544

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

White and green tea polyphenols inhibit pancreatic lipase *in vitro*

Anais Gondoin, Dominic Grussu, Derek Stewart, Gordon J. McDougall *

Plant Products and Food Quality Programme, Scottish Crop Research Institute Mylnefield, Invergowrie, Dundee DD2 5DA, Scotland, UK

ARTICLE INFO

Article history:
Received 18 February 2010
Accepted 29 April 2010

Keywords:
Tea
Polyphenols
Lipase
Fat digestion
Obesity
Health
Tannins

ABSTRACT

Green, white and black teas were assayed for inhibition of pancreatic lipase activity *in vitro*. White tea proved to be more effective than green tea with black tea showing little inhibition even at 200 μg GAE/ml. The EC_{50} values for inhibition were 22 μg/ml for white tea and 35 μg/ml for green tea; both easily achievable from normal infusions of tea. Liquid chromatography-mass spectroscopy analysis showed that white and green teas had essentially equal amounts of flavan-3-ols but green tea had higher levels of flavonols. White tea had higher levels of 5-galloyl quinic acid, digalloyl glucose, trigalloyl glucose and the tannin, strictinin.

After chromatography on Sephadex LH-20, the main inhibitory fraction was enriched in strictinin and fractions enriched in other components were ineffective. This suggests that strictinin content may be crucial for inhibition of pancreatic lipase. However, the possibility of synergies between the polyphenols cannot be disregarded.

1. Introduction

Tea is often quoted as being the most commonly consumed beverage in the world other than water. The majority of commercial teas arise from dried leaf material from *Camellia sinensis* L and a wide range of different teas can be produced. The main consumed types are black and green tea but recently white tea has become more available to consumers in the West. These teas mainly differ in their degree of processing but white tea is also generally composed only of the unopened bud and/or first leaves (Hilal & Engelhardt, 2007). After picking, white tea is dried and retains the white leaf hairs from which it derives its name. Green tea is heat-treated (steaming and/or pan-frying) to inactivate endogenous polyphenol oxidase (PPO), rolled and dried. At the other extreme, during black tea production there is no heat-inactivation of PPO and a "fermentation" or oxidation phase after rolling allows large-scale PPO-catalyzed conversion of simple phenolics to more complex forms and forms the dark coloration. As a result, white, green and black teas differ in their sensorial properties and have markedly different chemical compositions (e.g. Del Rio et al., 2004; Hilal & Engelhardt, 2007; Mizukami, Sawai, & Yamaguchi, 2007; Wang & Ho, 2009).

The major phenolics present in teas are the flavan-3-ols and the flavonols. The flavan-3-ols are characterized by (−)epicatechin and its galloylated derivatives, especially in green tea, whereas black tea has lower amount of these derivatives due to their oxidative conversion into theaflavins and thearubigins (Balentine, 1992; Del Rio et al., 2004; Finger, Kuhr, & Engelhardt, 1992; Mizukami et al., 2007). The flavonols are mainly derivatives of quercetin and kaempferol (Del Rio et al., 2004; Price, Rhodes, & Barnes, 1998) but there are smaller amounts of tannins and hydroxycinnamate derivatives. Of course, teas also contain substantial and physiological relevant levels of caffeine and theobromine (e.g. Roberts & Barone, 1983).

Epidemiological studies have suggested correlations of tea intake with favorable outcomes with regard to cardiovascular disease (Grassi et al., 2008), cancer incidence (Yang, Maliakal, & Meng, 2002), inflammation (Gonzalez de Mejia, Vinicio Ramirez-Mares, and Puangpraphant, 2009), obesity (Hsu & Yen, 2007; Wolfram, Wang, & Thielecke, 2006) and type 2 diabetes risk (Venables, Hulston, Cox, and Jeukendrup, 2008). Indeed, a range of mechanisms have been proposed for the beneficial effects of tea and health (e.g. Higdon and Frei, 2003), which largely focus on the polyphenol components, especially the flavan-3-ols.

Considering the epidemic of obesity now forecasted for the Western World (Anon, 2003), the possibility that tea intake could influence obesity through lipid metabolism and digestion is intriguing. Tea, and phenolic components of tea, have been suggested to have anti-obesity and anti-diabetic effects in humans (Kao, Chang, Lee, & Chen, 2006), to reduce adipose mass in rodent models (e.g. Kim et al., 2009) and to influence lipid digestion *in vitro* (Juhel et al., 2000). In this study we compare examples of black, green and white teas for their ability to inhibit pancreatic lipase *in vitro* and attempt to identify the active polyphenolic components.

* Corresponding author. Tel.: +44 1382 562426; fax: +44 1382 562731.
E-mail address: Gordon.mcdougall@scri.ac.uk (G.J. McDougall).

0963-9969/$ - see front matter
doi:10.1016/j.foodres.2010.04.029

2. Materials and methods

2.1. Tea extraction

Black tea (Tetley) and green tea (Clipper, Green China Tea) were purchased at a local supermarket and hand picked, loose leaf white tea was a gift [from Mr. White, Director of Honeybush & Butterbum Ltd, St Peter's Gate, Charles St, Sunderland SR6 0AN (www.t-please.com)]. The black and green teas were removed from their bags and weighed. An amount of loose white tea (~2 g) equivalent to the green tea was weighed into flasks. Triplicate tea samples were extracted with 200 ml of boiled water and incubated for 15 min at room temperature stirred with rotary shaking at 100 rpm. After cooling, the tea infusions were filtered through Whatman No. 1 filter paper and the recovery noted. The loose white tea had a larger particle size than the other teas.

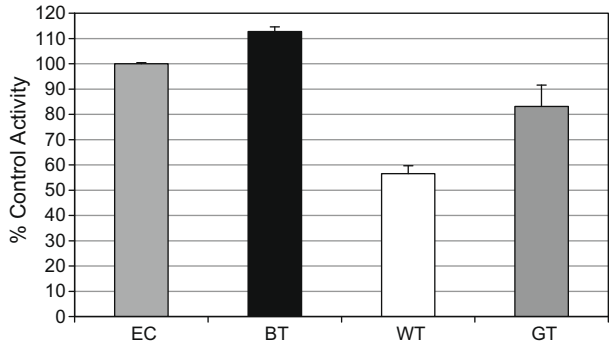

Fig. 1. Inhibition of lipase by tea infusions. Assays contained 25 μg GAE for white tea (WT) and green tea (GT) but contained 100 μg GAE for black tea (BT). EC is enzyme control, no inhibitors added. Values are expressed as % control activity and were triplicates ± standard errors.

Phenol content was measured using a modified Folin–Ciocalteu method (Deighton, Brennan, Finn, & Davies, 2000) and quantified as gallic acid equivalents (GAE). Samples were dried in aliquots of suitable phenol content in a Speed-Vac (Thermo Scientific, Basingstoke, UK).

A portion of each infusion was subjected to solid phase extraction (SPE) procedure used to gather phenol-rich fractions. Briefly, the infusion (50 ml) were applied to C18 solid phase extraction (SPE) units (Strata C18-E, GIGA units, 10 g capacity; Phenomenex Ltd., Macclesfield, UK) pre-washed in 0.1% (v/v) formic acid in acetonitrile then pre-equilibrated in 0.1% (v/v) formic acid in water. The unbound material was discarded. The SPE units were washed with a unit volume of 0.1% (v/v) aqueous formic acid then with two volumes of ultra-pure water. The polyphenol-enriched bound

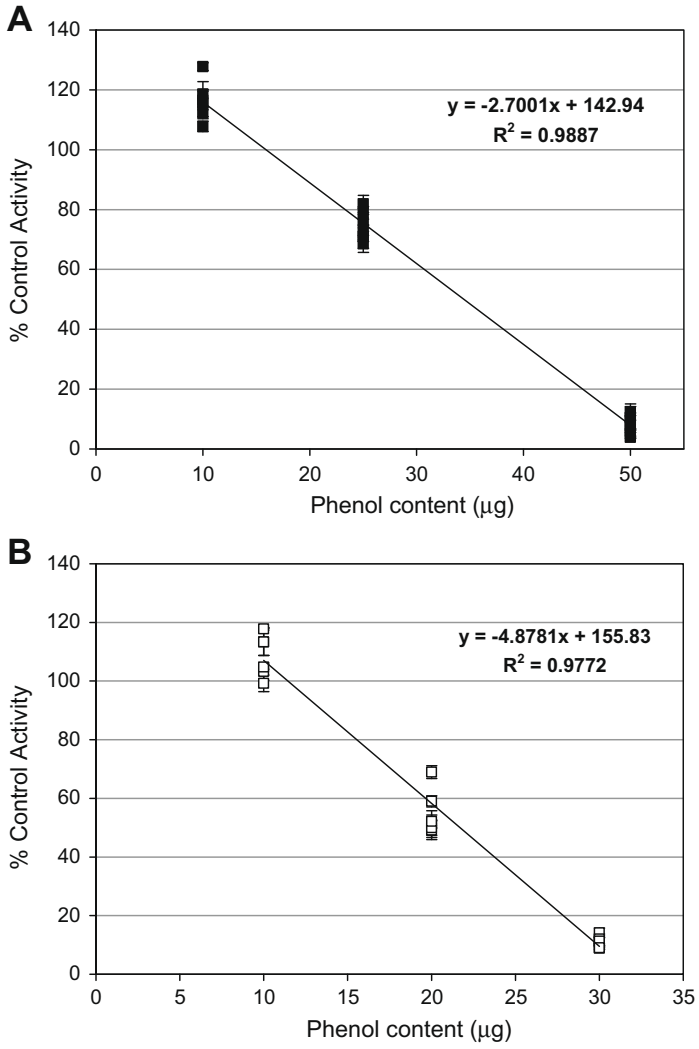

Fig. 2. Inhibition of lipase by green and white tea infusions. The dose response for green tea (A) and white tea (B) on lipase inhibition was assessed. Values are expressed as % control activity and were triplicates ±standard errors. Equations for the slopes assume straight lines relationships.

extracts eluted with acetonitrile. After measurement of phenol content, aliquots of the C18-bound extracts were evaporated to dryness in a Speed-Vac.

2.2. Chromatography on Sephadex LH-20

This procedure essentially followed previous work (McDougall et al., 2005). White tea infusion (50 ml) was diluted with an equal volume of ethanol and mixed by inversion. A small precipitate was removed by centrifugation (5000 g, 10 min, 5 °C) and a sample of the supernatant was removed for phenol content assessment and LC-MS analysis. The remainder was applied to a Sephadex LH-20 column which had been washed in 50% acetone/water then re-equilibrated in 50% ethanol/water. The unbound material that eluted during the application of the sample was collected then a wash fraction of 20 ml of 50% ethanol/water was applied and collected. The bound material was eluted in 3×20 ml fractions of 50% acetone. Each fraction was assayed for phenol content and aliquots of equivalent phenol content were dried for LC-MS and lipase inhibitor assay.

2.3. Lipase assay

This assay was as reported previously (McDougall, Kulkarni, & Stewart, 2009). 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.

The control assay contained 400 µl assay buffer, 450 µl substrate solution and 150 µl lipase. Tea samples were dissolved in ultra-pure water and added in 50 µl total volume. The buffer, enzyme and tea 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 1 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. The results are expressed as % control activity (McDougall et al., 2009).

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

Triplicate samples containing 20 µg phenols (gallic acid equivalents; GAE) were analyzed on an LCQ-Deca system, comprising Surveyor autosampler, pump and photodiode array detector (PDA) and a ThermoFinnigan ion-trap mass spectrometer. The PDA

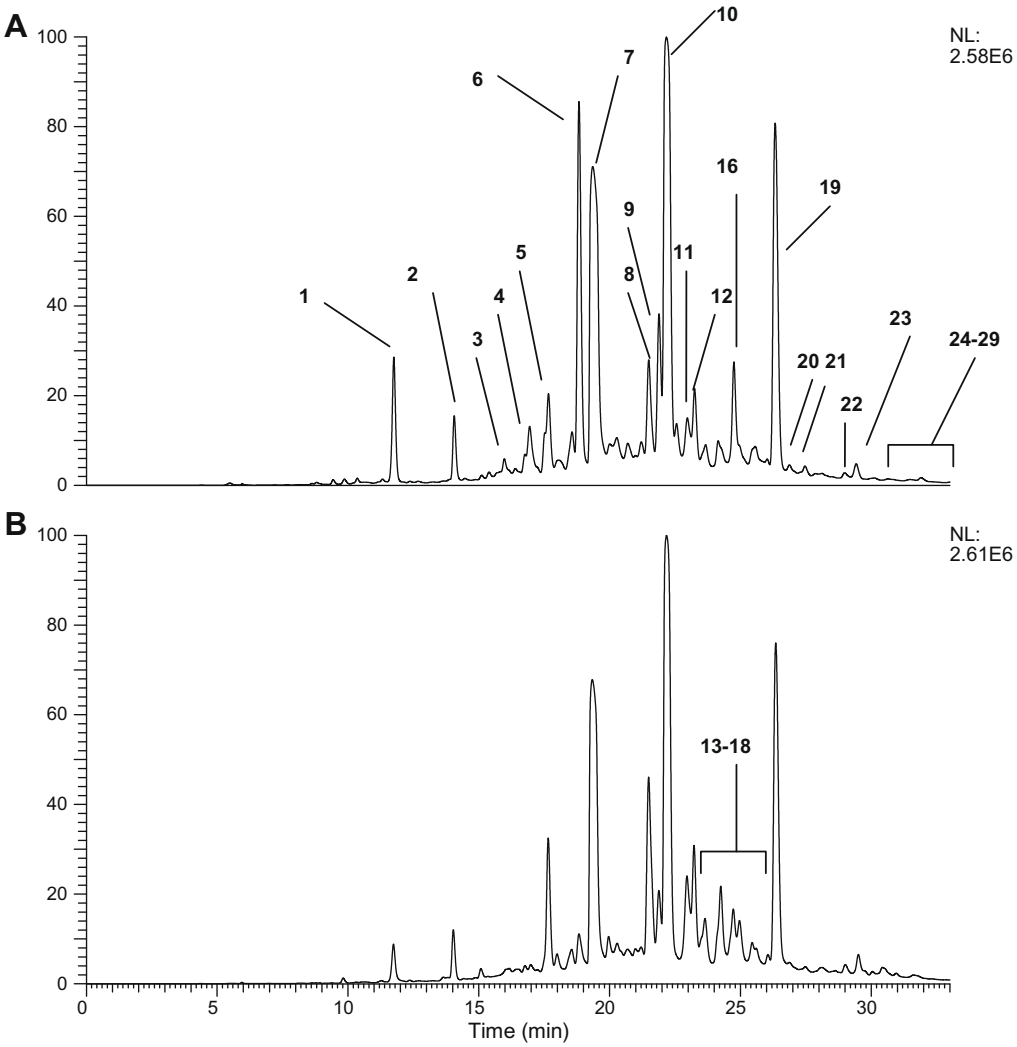

Fig. 3. LC-MS analysis of white and green tea infusions. Each trace (A280) is from 20 µg GAE. The figures in the top right corner represent the full scale deflection of the detector. Fig 3A is the white tea and (B) is the green tea extract. Peaks annotations match those in Table 1.

scanned discrete channels at 280 nm, 365 nm and 520 nm and recorded spectra from 200–600 nm. The samples were applied to a C18 column (Synergi Hydro C18 with polar endcapping, 4.6 mm × 150 mm, Phenomenex Ltd.) and eluted using a gradient of 5% acetonitrile (0.5% formic acid) to 40% 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 ionization) interface and analyzed 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 270 °C, with sheath gas at 60 psi and auxiliary gas at 10 psi. The MS was tuned against cyanidin-3-O-glucoside (positive mode) and (−)epicatechin in negative mode.

The relative amounts of polyphenols in white and green tea was estimated by searching for the major *m/z* value of the compound in question and calculating the peak areas using Xcalibur software. All estimations were carried out on LC-MS runs from three separate infusions. Peak areas were checked against PDA peak areas where possible.

3. Results

The black tea infusions yielded the highest phenol content (217 ± 0.9 mg GAE total) whereas white tea and green tea provided lower amounts (194 ± 1.4 and 176 ± 1.1 mg GAE total, respectively). However, because the infusions were made from the tea bags as supplied, the order was different when expressed as mg/g dry weight with white tea yielding the highest phenol content (97 ± 0.7 mg GAE/g) compared to 89.1 ± 0.5 (green tea) and 67.9 ± 0.3 (black tea). These values are in the same range as previous comparisons (e.g. ~100 mg/g; Rusak, Komes, Likić, Horžić, & Kovač, 2008).

White and green tea showed substantial inhibition of pancreatic lipase activity when screened at 25 μg GAE/ml. However, black tea showed no inhibition at 100 μg/ml (Fig. 1) and was ineffective at 200 μg/ml (results not shown). White tea was more effective than green tea with an EC_{50} value of 22 μg GAE/ml compared to 35 μg GAE/ml for green tea (Fig. 2a and b). Samples enriched in polyphenols obtained by solid phase extraction gave similar EC_{50} values of inhibition (results not shown). This strongly suggests that the inhibition was caused by the phenolic components. However, the order in which the components was added did not affect inhibition; i.e. preincubation of the enzymes with the tea samples was not essential for inhibition.

LC-MS analysis revealed that the green and white tea infusions had similar but subtly different phenolic compositions (compare Fig. 3a and b; Table 1). The green and white teas had similar levels of the flavan-3-ol derivatives; theobromine and caffeine (see Fig. 4). Green tea generally had higher levels of flavonols but there were major differences in the composition (Fig. 5). For example, green tea had higher levels of quercetin rutinoside (peak 15) and contained a range of quercetin and kaempferol tetraglycoside (Dou, Lee, Tzen, & Lee, 2008) derivatives (peaks 23–28) whereas white tea had higher levels of one kaempferol hexose rhamnose hexose derivative (peak 16). However, the main components which were higher in the white tea and could explain the higher lipase inhibition were identified as 5-galloyl quinic acid (3.2-fold), digalloyl glucose (7.1-fold), trigalloyl glucose (3.9-fold) and the tannin, strictinin (8.3-fold).

Chromatography on Sephadex LH-20 produced a range of unbound, wash and bound samples (Fig. 6). However, it should be

Table 1
Peak assignments in green and white tea infusions.

Peak No.	T_R	PDA	*M/Z* (M–H)	MS^2	Put. ID
1	11.75	275	**343**, 191	**191**, 169	5-galloyl quinic acid
2	14.06	270	181+	ND	Theobromine
3	15.08	275	305	261, 219, 179	Gallocatechin
4	16.94	270	**483**	423, 331, 271	Digalloyl glucose*
5	17.65	275	305	261, 219, 179	(−)epigallocatechin
6	18.83	265	**633**, 301	463, **301**, 275	Strictinin
7	19.34	260	195+	ND	Caffeine
7a	19.34	N.R	289	**245**, 205	(+)catechin
8	21.49	280	289	**245**, 205	Epicatechin
9	21.89	285	**635**	483, **465**, 313	Trigalloyl glucose
10	22.17	275	**457**, 305, 169	331, **305**, 169	EGCG
11	22.95	340, 280	593	413, 293	Theaflavin
12	23.22	350	**771**, 301	609, **301**	Quercetin hexose rhamnose hexose
13	23.64	340	577	403, 293	TF derivative
14	24.15	345	**755**, 285	593, 447, **285**	Kaempferol hexose rhamnose hexose
15	24.25	350	**609**, 301	301	Quercetin rutinoside
16	24.75	345	**755**, 285	593, **285**	Kaempferol hexose rhamnose hexose
17	25.45	350	**463**, 301	301	Quercetin hexose
18	26.03	345	**593**, 285	285	Kaempferol Rutinoside
19	27.14	350	**433**, 301	301	Quercetin pentose
20	27.47	345	**447**, 285	285	Kaempferol hexose
21	28.98	275	**441**, 289	289	ECG
22	29.01	275	**455**, 289	289	Methyl ECG
23	29.51	315 (270–400)	**1049**, 301	**903**, 885, 301	Quercetin hexose rhamnose coumaroyl pentose hexose
24	30.03	315 (280–400)	**1049**, 1033, 301	**903**, 885, 301	Quercetin hexose rhamnose coumaroyl pentose hexose
25	30.42	315 (280–400)	**887**, 917, 301	**741**, 301	Quercetin rhamnose coumaroyl pentose hexose
26	30.55	315 (280–400)	**917**, 301	**771**, 753, 301	Quercetin hexose rhamnose coumaroyl hexose
27	30.96	320 (280–400)	**887**, 301	**741**, 301	Quercetin rhamnose coumaroyl pentose hexose
28	31.62	320 (280–400)	**901**, 871, 755	Multiple	Kaempferol and quercetin derivatives
29	31.90	375, 440	563	Multiple	Theaflavin derivative

Peak annotations are shown on Figs. 3–6.
Figures in bold represent the main signal and the one chosen for MS^2 analysis. + denotes that the component ionized most readily in positive mode.
* = Three peaks containing a [*M* − *H*] signal at 483 could be discerned at RT 16.02, 16.94 and 17.57 suggesting the presence of isomers of digalloyl glucose (see Fig. 6). These are discussed in the results concerning fractionation on Sephadex LH-20.
White tea also contained detectable amounts of [*M* − *H*] signal at 785 (RT 22.59) which can be assigned to galloylated strictinin MS^2 = 633 [*M* − 152 = galloyl].
Identifications are supported by references (Del Rio et al., 2004; Dou et al., 2008; Mammela, Savolainen, Lindroos, Kanga, & Vartiainen, 2000).

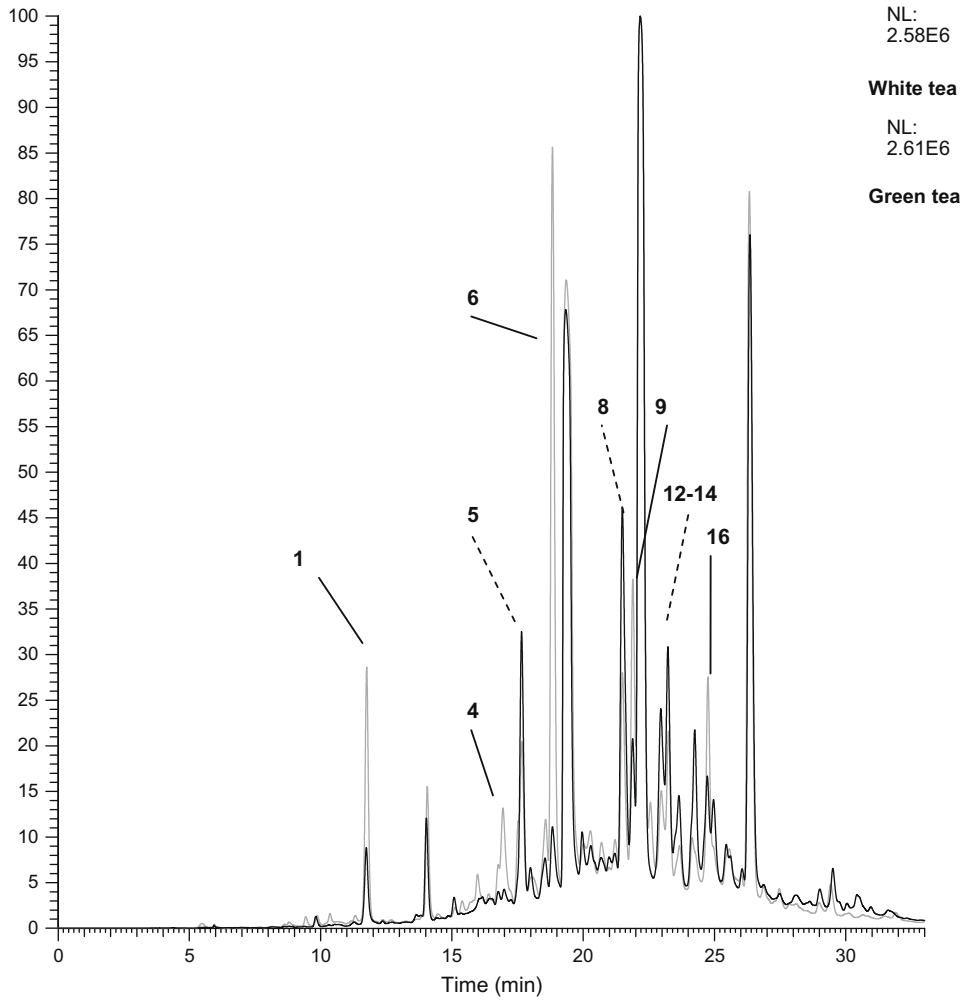

Fig. 4. LC-MS analysis of white and green tea infusions. The traces (A280) from white and green tea are overlaid to identify differences in phenolic content. The white tea trace is in grey and the green tea trace is black.

noted that diluting the white tea sample to 50% ethanol caused a marked change in the polyphenol composition (compare Fig. 6A and B); most obvious of which was the reduction in strictinin content (peak 6). We can only assume that some strictinin precipitated along with other components. The unbound fraction contained mainly caffeine (peak 7), theobromine (peak 2) and 5-galloyl quinic acid (peak 1) with smaller amounts of peaks (marked * in Fig. 6C), the first of which eluted where strictinin eluted, identified as *p*-coumaroyl quinic acid derivatives [PDA max at 310; *m/z* = 337, MS^2 191 and 163], which were concentrated in this fraction.

Due to carry-over, the wash fraction (Fig. 6D) also contained the peaks found in the unbound fraction but was also enriched in flavonol hexose rhamnose hexose derivatives (e.g. peaks 12, 14 and 16).

Bound fraction 1 (Fig. 6E) was enriched in (−)epigallocatechin, (+)catechin and epicatechin (peaks 5, 7a and 8) but also contained considerable amounts of three peaks that appear to be digalloyl glucose isomers (peaks 4 and 4*). The peak marked ** was gallic acid, which was not apparent in the original extract. This fraction also contained some strictinin (peak 6) and a range of flavonols derivatives (peaks 12, 14–20). Myricetin hexose, presumably myricetin-3-O-glucoside (Del Rio et al., 2004) was also detected in this fraction.

Bound fraction 2 was enriched in strictinin (peak 6) with smaller amounts of epicatechin and (−)epigallocatechin (peaks 5 and 8), digalloyl glucose (peak 4) and EGCG (peak 10). This fraction also contained trigalloyl glucose (peak 9, not shown on Fig. 6).

Bound fraction 3 was dominated by the catechin gallates (EGCG, peak 10; ECG, peak 21) with small amounts of methyl ECG (peak 22) but also contained strictinin (peak 6). Bound fraction 3 also contained another flavan-3-ol derivative not detected at first pass in the original white tea samples [at RT 22.95 with PDA max = 275; *m/z* = 425, 273 and MS^2 = 273, 169) which can be putatively identified as (epi)afzelechin gallate (Zeeb, Nelson, Albert, & Dalluge, 2000). This component was also present in the green tea infusions at roughly 3-fold lower levels than white tea but was overshadowed by flavonol derivatives (peaks 23 and 24).

The new white tea sample required 100 μg GAE/ml to give consistent lipase inhibition (Fig. 7) which may have been related to its apparently lower amounts of strictinin. However, the original white tea extract was also not so effective ($EC_{50} \sim 48$ μg/ml) at this point despite having an effectively identical polyphenol composition by LC-MS (results not shown). This reduction in effectiveness may have been the result of a new batch of lipase, which had higher protein content per unit of lipase activity. Therefore, although the lipase activity per mg weight was not affected, it had 1.5-fold higher protein content.

In any case, if 100 μg GAE/ml was used, it was clear that the bulk of the lipase inhibitory activity was recovered in bound fraction 2 with some activity in bound fraction 3 (Fig. 7).

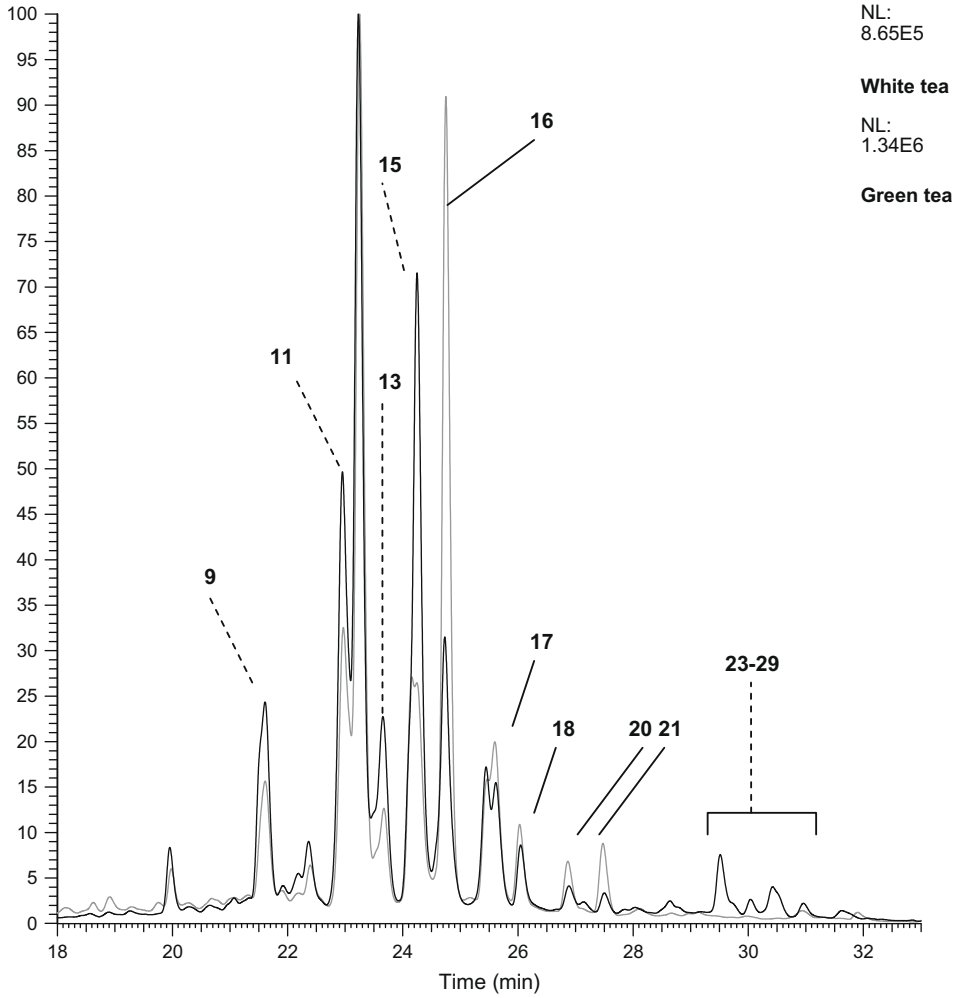

Fig. 5. LC-MS analysis of white and green tea infusions. The traces (A365) from white and green tea are overlaid to identify differences in phenolic content. The white tea trace is in grey and the green tea trace is black.

4. Discussion

The main difference between white and green tea was in the amounts of strictinin and digalloyl glucose derivatives. Fractionation on Sephadex LH-20 suggested that the strictinin content was most important for lipase inhibition. The variation in EC_{50} values noted between lipase batches was caused by variation in protein content which re-emphasizes the difficulty in comparing EC_{50} between studies or laboratories. The amelioration of inhibition by higher levels of non-enzymatic protein suggests that strictinin causes inhibition by binding to the lipase directly (Frazier et al., 2010). It is clear that more work to compare inhibition by purified compounds identified in this study or synergistic effects of mixtures of flavan-3-ols and tannins would be useful. However, this was not possible in this study but one could also argue that the value of using artificial mixtures of purified polyphenols when the commercially-available teas with different polyphenolic compositions are effective is debatable.

Previous work on lipase inhibition has focused on flavan-3-ol content and EGCG (Juhel et al., 2000). The lack of inhibition by black tea with its lower flavan-3-ol content generally supports this idea. However, it has been previously noted that strictinin and similar derivatives could inhibit lipase at low levels (Nakai et al., 2005).

Strictinin content and the general polyphenol composition of green tea (and probably white teas, although there is less data) varies with variety of tea, processing and location of growth (Engelhardt, Lakenbrink, & Pokorny, 2004; Komes, Horžić, Belščak, Kovačević, & Vulić, 2010; Lin, Tsai, Tsay, and Lin, 2003). Also different polyphenols are differently influenced by the means of preparing tea beverages (Mizukami et al., 2007; Rusak et al., 2008; Yang, Hwang, & Lin, 2007) and subsequent storage (Friedman, Levein, Choi, Kozukue, & Kozukue, 2006; Mizukami et al., 2007). Indeed, tea flavan-3-ols can undergo epimerization reactions on heating (Seto, Nakamura, Nanjo, & Hara, 1997) which may influence their stability and bioactivity (Ikeda et al., 2003). In this study, we noted that strictinin in white tea preparations was substantially less stable in frozen aqueous solution than when reconstituted from freeze-dried powder or from frozen speed-vac dried samples (results not shown).

It has also become clear that tea polyphenols may have different stabilities under simulated gut conditions (e.g. Record and Lane, 2001) and may form other products during digestion. However, although it is clear that they are present in the gut in sufficient amounts to inhibit lipase (Stalmach et al., 2009), it is not known if they are active *in vivo*.

Ingestion of green tea has been shown to have blood lipid and cholesterol lowering effects in animal models fed high fat diets (e.g. Ikeda et al., 1992, 2003). Systemic effects on lipid metabolism have been identified (e.g. Hsu & Yen, 2007; Kim et al., 2009) and tea ingestion increases serum antioxidant levels (Serafini, Ghiselli, & Ferro-Luzzi, 1996). However, due to the low serum bioavailability of tea polyphenols (e.g. Henning et al., 2005), it has been sug-

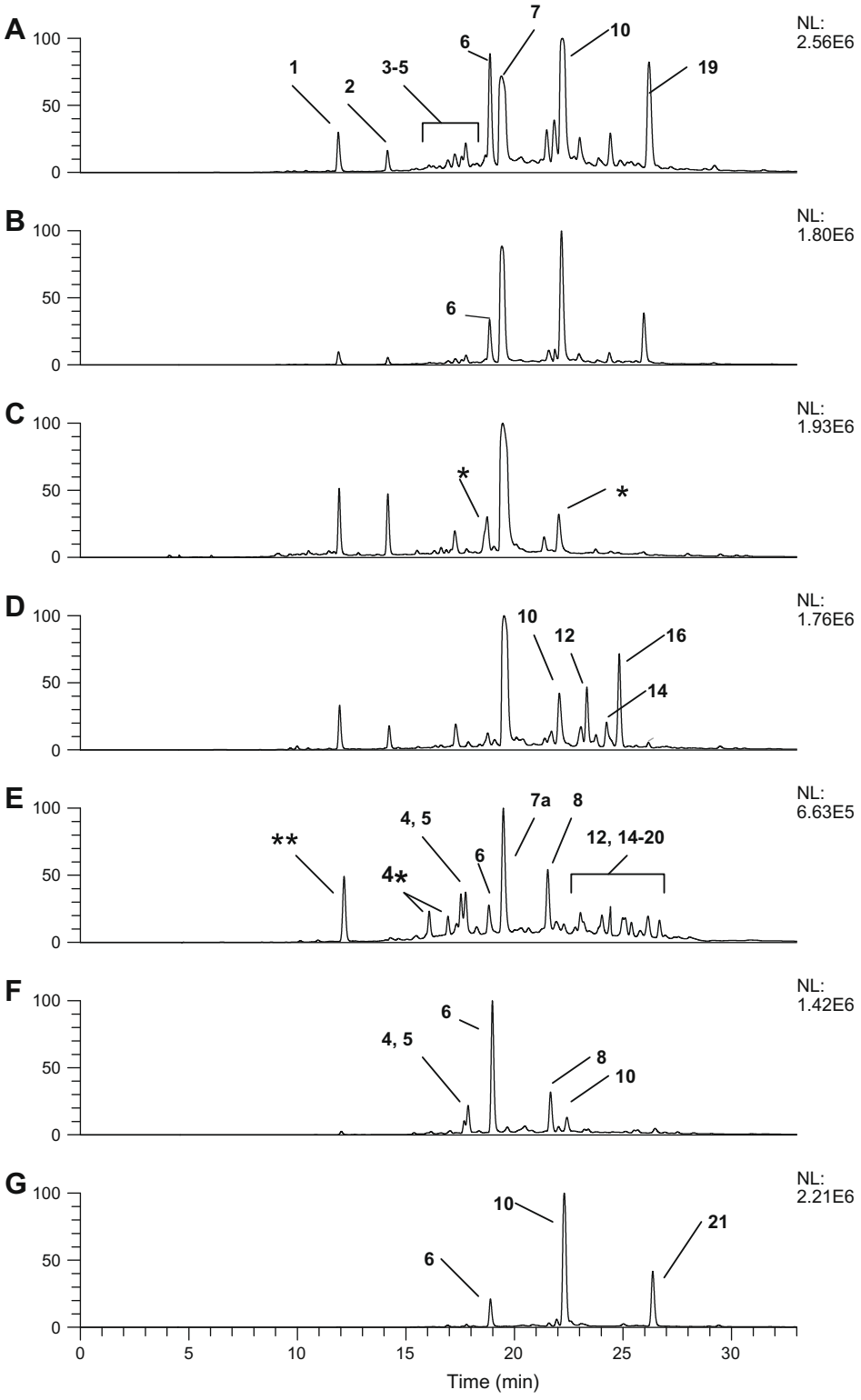

Fig. 6. LC-MS analysis of fractions from separation of white tea on Sephadex LH-20 Equal amounts (20 μg GAE) of fractions from the Sephadex LH-20 were applied to LC-MS and traces at 280 nm are shown. A = the original white tea infusion; B = white tea after treatment with 50% ethanol; C = the unbound fraction; D = the wash fraction; E = the bound fraction 1; F = bound fraction 2 and G = bound fraction 3. Figures in the right corners are full scale deflections. Peaks annotations refer to Table 1 or are discussed in the text.

gested that effects on lipid levels *in vivo* must be "mediated largely via their influence on the intestinal processes involved in digestion and absorption of lipids" (Koo & Noh, 2007). Inhibition of lipase and subsequent slowing of fat digestion could be one of a range of mechanisms whereby tea polyphenols could influence obesity. Work has largely focused on EGCG and related compounds and has suggested that as well as inhibition of lipases, the main effect of these compounds may be to interfere with the emulsification and micellar solubilization of lipids and thereby reduce their overall uptake (Koo & Noh, 2007). However, it seems entirely possible that teas with elevated strictinin levels could increase inhibition of lipase and act synergistically with the flavan-3-ols

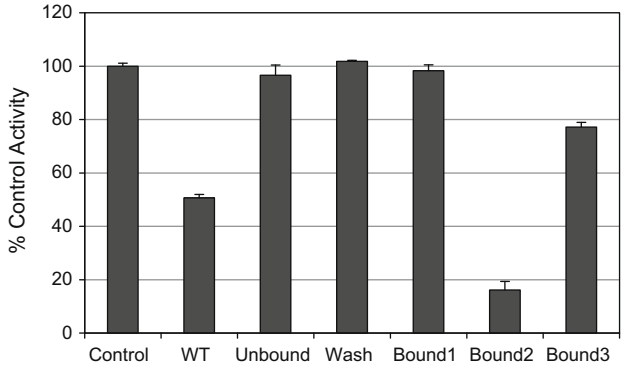

Fig. 7. Inhibitory activity of LH-20 fractions. Lipase inhibition assays were carried out at 100 μg GAE. Values are expressed as % control activity and were triplicates ±standard errors.

to increase the overall lipid-lowering effect. This synergy may explain the greater lipid-lowering effectiveness of green tea extracts over EGCG alone (e.g. Koo & Noh, 2008). Such beverages could find use as near-natural drugs to control lipid levels and obesity (Birari & Bhutani, 2007).

Acknowledgements

We acknowledge the gift of white tea from Honeybush & Butterbum Ltd, via Dr Ed Okello, University of Newcastle. We also thank the École Nationale Supérieure d'Agronomie et des Industries Alimentaires, Nancy, France for allowing Anais Gondoin to spend her internship training period at SCRI. We also thank the Rural and Environment Research and Analysis Directorate, Scottish Government for support.

References

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

Balentine, D. (1992). Manufacturing and chemistry of tea. In: C.-T. Ho, T. Osawa, M.-T. Huang, R.T. Rosen (Eds.), *Phenolic compounds in food and their effects on health* (pp. 102–117) Washington, DC: American Chemical Society.

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

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.

Del Rio, D., Stewart, A. J., Mullen, W., Burns, J., Lean, M. E. J., Brighenti, F., et al. (2004). HPLC-MSn analysis of phenolic compounds and purine alkaloids in green and black tea. *Journal of Agricultural and Food Chemistry, 52*, 2807–2815.

Dou, J., Lee, V. S. Y., Tzen, J. T. C., & Lee, M.-R. (2008). Rapid identification of acylated flavonol tetraglycosides in oolong teas using HPLC-MSn. *Phytochemical Analysis, 19*, 251–257.

Engelhardt, U. H., Lakenbrink, C., & Pokorny, O. (2004) Proanthocyanidins, bisflavanols, and hydrolyzable tannins in green and black teas. In: F. Shahidi, D. K. Weerasinghe (Eds.), Nutraceutical beverages. Chemistry, nutrition, and health effects. ACS symposium series, vol. 871, pp. 254–264.

Finger, A., Kuhr, S., & Engelhardt, U. H. (1992). Chromatography of tea constituents. *Journal of Chromatography, 624*, 293–315.

Frazier, R. A., Deaville, E. R., Green, R. J., Stringano, E., Willoughby, I., Plant, J., et al. (2010). Interactions of tea tannins and condensed tannins with proteins. *Journal of Pharmaceutical and Biomedical Analysis, 51*, 490–495.

Friedman, M., Levein, C. E., Choi, S.-H., Kozukue, E., & Kozukue, N. (2006). HPLC analysis of catechins, theaflavins, and alkaloids in commercial teas and green tea dietary supplements: Comparison of water and 80% ethanol/water extracts. *Journal of Food Science, 71*, 328–337.

Gonzalez de Mejia, E., Vinicio Ramirez-Mares, M., & Puangpraphant, S. (2009). Bioactive components of tea: Cancer, inflammation and behavior. *Brain, behavior and Immunity, 23*, 721–731.

Grassi, D., Aggio, A., Onori, L., Croce, G., Tiberti, S., Ferri, C., et al. (2008). Tea, flavonoids, and nitric oxide-mediated vascular reactivity. *Journal of Nutrition, 38*, 1554S–1560S.

Henning, S. M., Niu, Y. T., Liu, Y., Lee, N. H., Hara, Y., Thames, G. D., et al. (2005). Bioavailability and antioxidant effect of epigallocatechin gallate administered in purified form versus as green tea extract in healthy individuals. *Journal of Nutritional Biochemistry, 16*, 610–616.

Higdon, V., & Frei, B. (2003). Tea catechins and polyphenols: Health effects, metabolism, and antioxidant functions. *Critical Reviews in Food Science and Nutrition, 43*, 89–143.

Hilal, Y., & Engelhardt, U. (2007). Characterization of white tea–comparison to green and black tea. *Journal für Verbraucherschutz und Lebensmittelsicherheit, 2*, 414–421.

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.

Ikeda, I., Imasato, Y., Sasaki, E., Nakayama, M., Nagao, H., Takeo, T., et al. (1992). Tea catechins decrease micellar solubility and intestinal absorption of cholesterol in rats. *Biochimica et Biophysica Acta, 1127*, 141–146.

Ikeda, I., Kobayashi, M., Hamada, T., Tsuda, K., Goto, H., Imaizumi, K., et al. (2003). Heat-epimerized tea catechins rich in gallocatechin gallate and catechin gallate are more effective to inhibit cholesterol absorption than tea catechins rich in epigallocatechin gallate and epicatechin gallate. *Journal of Agricultural and Food Chemistry, 51*, 7303–7307.

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.

Kao, Y. H., Chang, H. H., Lee, M. J., & Chen, C. L. (2006). Tea, obesity, and diabetes. *Molecular Nutrition & Food Research, 50*, 188–210.

Kim, H. J., Leon, S. M., Lee, M. K., Jung, U. J., Shin, S. K., & Choi, M. S. (2009). Anti-lipogenic effect of green tea extract in C57BL/6JLep ob/ob mice. *Phytotherapy Research, 23*, 467–471.

Komes, D., Horžić, D., Belščak, A., Kovačević, G. K., & Vulić, I. (2010). Green tea preparation and its influence on the content of bioactive compounds. *Food Research International, 43*, 167–176.

Koo, S. I., & Noh, S. K. (2007). Green tea as inhibitor of the intestinal absorption of lipids: Potential mechanism for its lipid-lowering effect. *Journal of Nutritional Biochemistry, 18*, 179–183.

Koo, S. I., & Noh, S. K. (2008). Green tea extract is more effective then epigallocatechin gallate (EGCG) and epicatechin (EC) in inhibiting the lymphatic absorption of cholesterol (CH) and other lipids. *FASEB Journal, 22*, 701–708.

Lin, Y. S., Tsai, Y. J., Tsay, J. S., & Lin, J. K. (2003). Factors affecting the levels of tea polyphenols and caffeine in tea leaves. *Journal of Agricultural and Food Chemistry, 51*, 1864–1873.

Mammela, P., Savolainen, H., Lindroos, L., Kanga, J., & Vartiainen, T. (2000). Analysis of oak tannins by liquid chromatography-electrospray ionisation mass spectroscopy. *Journal of Chromatography A, 891*, 75–83.

McDougall, G. J., Kulkarni, N. N., & Stewart, D. (2009). Berry polyphenols inhibit pancreatic lipase activity in vitro. *Food Chemistry, 115*, 193–199.

McDougall, G. J., Shpiro, F., Dobson, P., Smith, P., Blake, A., & Stewart, D. (2005). Different polyphenolic components of soft fruits inhibit α-amylase and α-glucosidase. *Journal of Agricultural and Food Chemistry, 53*, 2760–2766.

Mizukami, Y., Sawai, Y., & Yamaguchi, Y. (2007). Simultaneous analysis of catechins, gallic acid, strictinin, and purine alkaloids in green tea by using catechol as an internal standard. *Journal of Agricultural and Food Chemistry, 55*, 4957–4964.

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.

Price, K. R., Rhodes, M. J. C., & Barnes, K. A. (1998). Flavonol glycoside content and composition of tea infusions made from commercially available teas and tea products. *Journal of Agricultural and Food Chemistry, 46*, 2517–2522.

Record, I. R., & Lane, J. M. (2001). Simulated intestinal digestion of green and black teas. *Food Chemistry, 73*, 481–486.

Roberts, H. R., & Barone, J. J. (1983). Biological effects of caffeine—history and use. *Food Technology, 37*, 32–39.

Rusak, G., Komes, D., Likić, S., Horžić, D., & Kovač, M. (2008). Phenolic content and antioxidative capacity of green and white tea extracts depending on extraction conditions and the solvent used. *Food Chemistry, 110*, 852–858.

Serafini, M., Ghiselli, A., & Ferro-Luzzi, A. (1996). In vivo antioxidant effect of green and black tea in man. *European of Journal Clinical Nutrition, 50*, 28–32.

Seto, R., Nakamura, H., Nanjo, F., & Hara, Y. (1997). Preparation of epimers of tea catechins by heat treatment. *Bioscience, Biotechnology, and Biochemistry, 61*, 1434–1439.

Stalmach, A., Mullen, W., Steiling, H., Williamson, G., Lean, M. E., & Crozier, A. (2009). Absorption, metabolism, and excretion of green tea flavan-3-ols in humans with an ileostomy. *Molecular Nutrition and Food Research, 54*, 323–334.

Venables, M. C., Hulston, C. J., Cox, C. R., & Jeukendrup, A. E. (2008). Green tea extract ingestion, fat oxidation, and glucose tolerance in healthy humans. *American Journal Clinical Nutrition, 87*, 778–784.

Wang, Y., & Ho, C.-T. (2009). Polyphenolic chemistry of tea and coffee: A century of progress. *Journal of Agricultural and Food Chemistry, 57*, 8109–8114.

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.

Yang, D.-J., Hwang, L. S., & Lin, J.-T. (2007). Effects of different steeping methods and storage on caffeine, catechins and gallic acid in bag tea infusions. *Journal of Chromatography A, 1156*, 312–320.

Yang, C. S., Maliakal, P., & Meng, X. (2002). Inhibition of carcinogenesis by tea. *Annual Review of Pharmacology and Toxicology, 42*, 25–54.

Zeeb, D. J., Nelson, B. C., Albert, K., & Dalluge, J. J. (2000). Separation and identification of twelve catechins in tea using liquid chromatography/atmospheric pressure chemical ionization-mass spectrometry. *Analytical Chemistry, 72*, 5020–5026.