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Flavonoid recovery and stability from *Ginkgo biloba* subjected to a simulated digestion process

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Abstract

Absorption from the diet is normally a prerequisite for the potential in vivo beneficial role of flavonoids. Antioxidant activity of flavonoids in vitro has been the subject of several studies, and important structure–activity relationships of the antioxidant activity have been established. However, there is still debate about the stability and absorption of polyphenols under gastrointestinal conditions. *Ginkgo biloba*, a product well known for its flavonoid content, was chosen for this study. *Ginkgo biloba* leaves, standardised leaf extract EGb 761 and commercial tablets (containing EGb761) were incubated in simulated gastrointestinal fluids to determine the stability of their flavonoid profiles. The experiment was designed to mimic the human gut condition. HPLC analysis was then conducted to determine the resulting breakdown compounds and intact flavonoids after the incubation, thus indicating those compounds likely to be available for absorption. The different samples seem to react differently to the simulated digestion process. The results indicate a trend of conversion from the glycosides to the aglycones for some samples and subsequent degradation of the aglycones. This may indicate a need to further investigate the reported benefits of Ginkgo flavonoids as in vivo antioxidants and/or to consider the antioxidant activity of the resulting digestion-derived compounds.

Keywords: *Ginkgo biloba* leaves; *Ginkgo biloba* commercial capsules; Simulated gastrointestinal fluids; EGb 761; Flavonoids; HPLC

1. Introduction

The fate of phytochemicals in *Ginkgo biloba* leaves and their extract (EGB761) (DeFeudis, 1998) in the human body after ingestion is of interest in order to investigate the potential role of flavonoids as regards their radical-scavenging ability. Over recent years, *Ginkgo biloba* has achieved unprecedented popularity, as a health supplement. Concurrently, a major thrust of research has been the development of new food products, which have functional properties, which are truly expressed in the body.

An experiment (in vitro) to simulate the workings of the human gut during digestion was designed to study the recovery and stability of the flavonoids. The products of interest were raw *Ginkgo biloba* leaves, commercial *Ginkgo biloba* leaf extract capsules and standardised *Ginkgo biloba* extract. These products were subjected to conditions that closely followed those encountered in the gastrointestinal tract.

The aim of this study was to achieve a better understanding of what happens to the flavonoid compounds when they are placed in the mouth, carried by peristalsis to the stomach and the fate of these compounds when they come into contact with the upper gut secretions.

It has been reported that flavonoids present in foods cannot be absorbed from the intestine because they are bound to sugars as glycosides (Kuhnau, 1976). Only free flavonoids without a sugar molecule, the so-called aglycones, are considered to be able to pass through the gut wall, and no enzymes that can split these predominantly β-glycosidic bonds are secreted into the gut or present in the intestinal wall. However, in contrast, Hollman and his team (1999) suggest that, certainly for quercetin glycosides from onions, the absorption of the intact glycosides is in fact far better then the pure aglycones. These workers suggest that absorption

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kinetics and bioavailability of the flavonoids are probably governed by the form of glycoside present. However, this current work would seem to support the earlier report by Paganga and Rice-Evans (1997) who showed the presence of glycosylated flavonoids present in human plasma, in that the digested raw leaf residues were high in glycoside content.

This study aims to investigate the stability of flavonoid compounds before considering whether they are absorbed. If the aglycones or their glycosides were totally degraded in the gastrointestinal fluids then their absorption would be impossible. This simulated digestion/absorption study was undertaken with two objectives: (a) to determine the degree of degradation, if any, in the upper GI tract and (b) in view of the difficulty of measuring flavonoids in the blood, and being able to relate such levels to specific foods in the diet, to assess the availability of specific flavonoids for absorption. An in vitro study allows a more controlled approach to test hypotheses, which can later be studied, in vivo. Other studies have been carried out to determine flavonols in body fluids and these generally seem to indicate minimal degradation, at least for quercetin or its glycosides with gastrointestinal fluids (Hollman, de Varies, van Leeuwen, Mengelers, & Katan, 1995).

This study aims to provide further information on the stability of flavonoids in the human organism during the digestion process and to contribute to the development of *Ginkgo biloba* leaf products, which can later be directed to consumers with an increased knowledge of the content and biological activity of these natural antioxidants.

2. Materials and methods

2.1. Materials

The dried *Gingko biloba* leaves used were imported from Ventura County, California, USA. *Ginkgo biloba* leaf commercial capsules (claimed to be dried leaves) were purchased from a local General Nutrition Center. Each capsule is claimed to contain 50 mg of G*inkgo biloba* leaf extract, which has been ground to a fine powder. Standardised *Ginkgo biloba* leaf extract (EGb761) used was purchased from Voigt Global Distribution Kansas City, USA. These three samples were used to determine the flavonoid profiles based on their stability in the gastrointestinal fluids.

Authentic samples of quercetin-3-O-rutinoside (rutin), quercetin-3-galactoside, kaempferol-3-rutinoside, quercetin-3-rhamnoside (quercetrin), kaempferol-3-O-rutinoside, kaempferol-3-(*p*-coumaryl) glycoside], were obtained from Apin chemical, Abingdon, UK. Quercetin, kaempferol, and isorhamnetin standards were purchased from Aldrich Chemical Co. Reference solutions were prepared in methanol (varying concentration from 100 to 500 ppm). Pepsin, monobasic potassium phosphate, pancreatin mix, NaOH, NaCl and HCl, used for the simulation of the gastrointestinal fluids, were purchased from Sigma Aldrich, Singapore. 2-Propanol and tetrahydrofuran (THF), used for chromatography, were of HPLC grade.

2.2. Ginkgo biloba leaves preparation

The leaves were first ground for 20 s, a particle size distribution of 98% being retained in the 177 μm sieve. The size of the leaf particles is 98% larger than 177 μm but 88% smaller than 354 μm. Sample extractions (w/v 1:10) were carried out with two different solvents, i.e. water and ethanol/water (80:20), via stirring overnight. These samples were used as controls for the profile analysis. Prior to injection into the HPLC for the compounds analysis (20 μl), the samples were filtered through a 0.45 μm membrane filter.

2.3. Particle size determination

Particle size determination was performed on Ginkgo leaves using Tyler Standard Screen Scale Sieves (Cadle, 1955). Particle size and particle size distributions are generally measured because of the relationships they bear to other properties of such particles (Cadle, 1955). In this case, particle size determination is used to set the baseline on the effect of release of antioxidant compounds during extraction.

2.4. Preparation of simulated gastric fluids and intestinal fluid

Simulated gastric fluid was prepared according to the procedure of the USP, National Formulary: 2.0 g NaCl, 3.2 g pepsin and 3.0 ml concentrated HCl. diluted to 1 l and verified that the pH is 1.2–1.8.

Simulated intestinal fluid was prepared according to the procedure of the USP, National Formulary: 6.8 g monobasic potassium phosphate, 650 ml water, 190 ml of 0.2 mol/l NaOH and pancreatin mix (10 g).

In this experiment the simulated gastric juice, was made up as above and incubation conditions with temperature control and vortex action were applied to mimic the stomach conditions. The temperature was set at 37 °C and the churning action of the stomach was mimicked by vortexing the chyme at 100 rpm. In vivo, after residing in the stomach for 1–2 h, the chyme then proceeds on to the small intestine and for this experiment intestinal fluids were made up to mimic the pancreatic fluids and allowed to react for 2–4 h in a vortexing bath, at 37 °C, corresponding to the average and maximal transit time in the small intestine, respectively.

2.5. Incubation of Ginkgo biloba leaves in simulated gastrointestinal fluids

Carried out in triplicate, 1 g of *Ginkgo biloba* leaves was incubated with the simulated gastric juice at 37 °C for 1 h (designated step 1). In step 2, the earlier amounts from the simulated gastric juice (chyme) were then incubated with the intestinal fluid at 37 °C, for 2 and 4 h, respectively. These steps were carried out so as to mimic the digestion process as discussed earlier. The incubated samples from both steps, 1 and 2, were then filtered and made up to 25 ml. A duplicate of the supernatant from both steps was shaken with methanol to extract the alcohol soluble compounds. From this, two samples were obtained, namely an aqueous acidic extract and an alcoholic acid extract. Both solvent systems were used in order to track possible changes in the flavonoids as aglycones are more likely to be found in the alcohol medium whilst the glycosides are more likely to be associated with the aqueous medium. This allowed comparison with the undigested samples. This experiment yields the range of samples shown in Table 1.

2.6. Incubation of Ginkgo biloba leaf commercial capsules and standardised Ginkgo biloba leaf extract (EGb761) in simulated gastrointestinal fluids

Similar steps, as detailed in 2.4, were carried out on the commercial capsules but using 2 capsules in 25 ml and for EGb 761, using 0.25 g in 25 ml. This was deemed a realistic ratio of what might occur in vivo.

2.7. Chromatographic conditions

An HPLC system (Shimadzu HPLC, Shimadzu, Kyoto, Japan), equipped with a photo-diode array detector, was used and detection of the flavonoids was carried out at 360 nm. The separation was performed on a Shim-Pack VP-ODS column (250×4.6 mm i.d.) (Shimadzu, Kyoto, Japan) using mobile phase A: water: 2-propanol (95:5), eluent B: 2-propanol: THF: water (40:10:50), binary gradient from 20% to 55.2% B in 44 min at a flow-rate of 1.0 ml/min at 40 °C. The solvent was filtered through a 0.45 μm membrane filter and degassed using an ultrasonic bath or by flushing with helium before use. The mobile phase conditions were modified from a method previously reported by Pietta et al. (1991). This method is capable of analyzing 8 flavonoids in the various samples during one HPLC analysis.

2.8. Identification and determination of the glycosides and aglycones

The identification of the HPLC chromatographic peaks was established with external standards. Each sample was injected three times for HPLC profiling. The linearity of the determination of the 5 flavonol glycosides and 3 flavonol aglycones was verified by linear regression. The correlation coefficients were 0.9446 for rutin, 0.999 for quercetin-3-galactosides, 0.998 for kaempferol-3-rutinoside, 0.997 for quercetin-3-O-rhamnoside, 0.997 for quercetin, 0.998 for isorhamnetin and 0.998 for kaempferol. Standards, such as quercetin, isorhamnetin and kaempferol, used for calibration, are

Table 1
Experimental samples obtained from standardised *Ginkgo biloba* extract, *Ginkgo biloba* leaves extract and *Ginkgo biloba* capsules

Type of samples and treatment[a]		Description	Sample code
Step 1	Step 2		
Samples in aqueous extract left overnight	–	Control	$Blank_{aqueous}$
Samples in alcohol extract left overnight	–	Control	$Blank_{alcohol}$
Samples incubated with gastric juice 1 h in aqueous extract	–	To mimic food residing in the stomach condition for 1 h	$A_{aqueous}$
Samples incubated with gastric juice 1 h in alcohol extract	–	To mimic food residing in the stomach condition for 1 h	$B_{alcohol}$
Samples incubated with gastric juice 1 h	Followed by incubation in intestinal fluid for 2 h in aqueous extract	To mimic food residing in the stomach for 1 h then passing through the intestinal fluids for 2 h	$C_{aqueous}$
Samples incubated with gastric juice 1 h	Followed by incubation in intestinal fluid for 2 h in alcohol extract	To mimic food residing in the stomach for 1 h then passing through the intestinal fluids for 2 h	$D_{alcohol}$
Samples incubated with gastric juice 1 h	Followed by incubation in intestinal fluid for 4 h in aqueous extract	To mimic food residing in the stomach for 1 h then passing through the intestinal fluids for 4 h	$E_{aqueous}$
Samples incubated with gastric juice 1 h	Followed by incubation in intestinal fluid for 4 h in alcohol extract	To mimic food residing in the stomach for 1 h then passing through the intestinal fluids for 4 h	$F_{alcohol}$

[a] Samples were prepared and analysed in triplicate.

reported to have a very high stability with a loss of <4% within 12 months after storage at 8 °C in a refrigerator (Hasler & Sticher, 1992).

2.9. Purity assay of the chromatographic peaks

The UV spectra of each peak, after subtraction of the corresponding UV base spectrum of the standards, was normalised by the computer and their plots superimposed. Peaks were considered to be similar when there was exact matching among the corresponding standard spectra and also by comparison of retention times in both standards and samples.

3. Results and discussion

3.1. General

The constituents of various *Ginkgo biloba* samples were examined and 5 flavonoid glycosides and 3 aglycones were positively identified as shown in Fig. 1. These peaks were identified by comparison with the authentic standards. Together with the retention time data, these peaks were also identified by comparing their UV spectra with those of the corresponding standards.

Table 2 shows the amount of constituents identified from the various control samples, i.e. before incubation. From these results, which were designated 100%, an increase or decrease in the percentage after the incubation procedures was obtained. Tables 3–5 show results for the different samples.

3.2. Raw Ginkgo leaves

The $blank_{aqueous}$ and $blank_{alcohol}$ extract of raw Ginkgo leaves gave different values in the determined amounts of the various constituents. The difference between the two extracts provides an understanding on the extraction power of different solvents and also verifies the nature of the compounds of interest. In the aqueous extraction of raw leaves, flavonol aglycones could not be detected and there was a lower extraction of the flavonoid glycosides than with the alcohol extract (Table 2).

However, following incubation of the raw leaves with gastric fluids for 1 h and subsequent intestinal fluids for 2 and 4 h, respectively, the aqueous extract seems to display an increase in the amounts of compounds identified (Table 3). Flavonoids are ubiquitous and are found in the flowers, fruits and leaves of the *Ginkgo biloba* plant. They are biosynthesised from the starting material glucose via the pathway reported by Haslam (1993). Plant cells walls are mainly composed of linear chains of covalently linked glucose residues. They are very stable chemically and extremely insoluble.

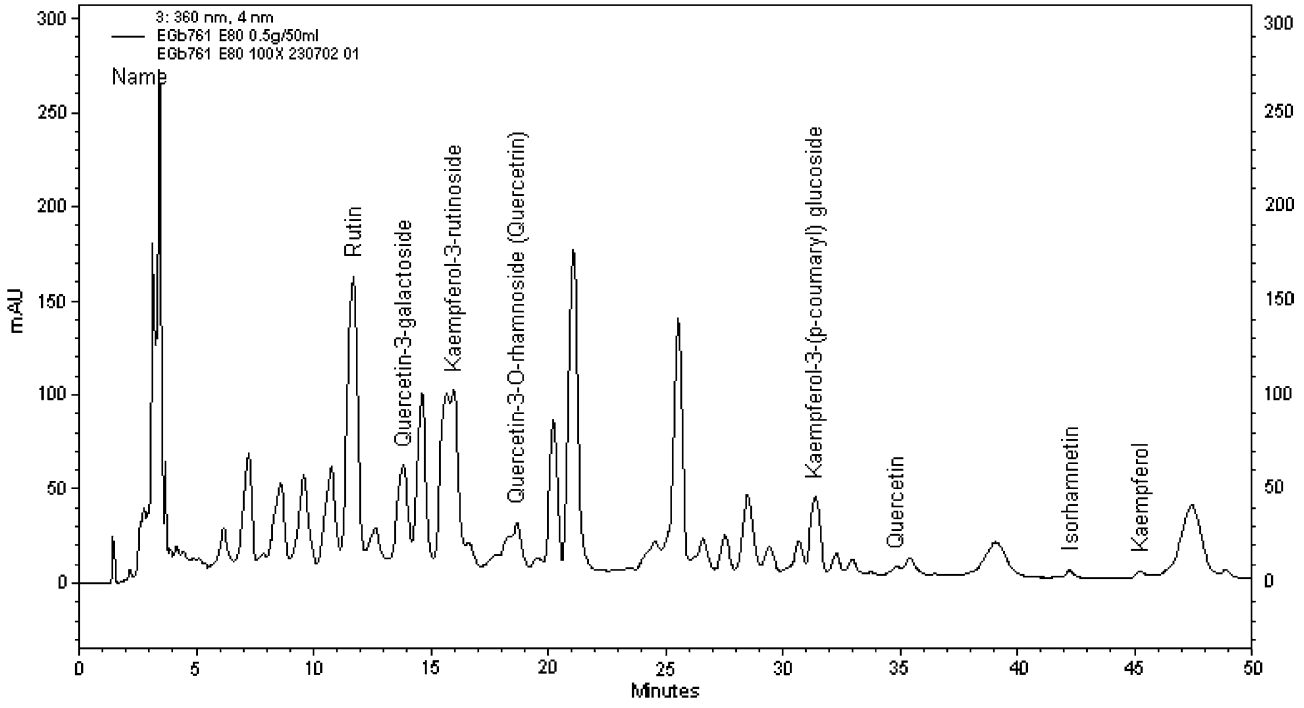

Fig. 1. Chromatogram of a standardised extract of *Ginkgo biloba* leaves (control sample: $blank_{alcohol}$). The constituents determined are quercetin-3-O-rutinoside (rutin), quercetin-3-galactoside, kaempferol-3-rutinoside, quercetin-3-rhamnoside (quercetrin), kaempferol-3-O-rutinoside, kaempferol-3-(*p*-coumaryl) glycoside, quercetin, kaempferol, and isorhamnetin. Shim-Pack VP-ODS column (250 × 4.6 mm i.d.); Mobile Phase A: water: 2-propanol (95:5), eluent B: 2-propanol: THF: water (40:10:50), binary gradient from 20% to 55.2% B in 44 min at a flow-rate of 1.0 ml/min at 40 °C.

Table 2
Comparison of the amount of the various constituents (mg/g) present in the various *Ginkgo biloba* samples studied, both in the aqueous and alcohol extracts

Type of samples	Recovered amount (mg/g) of the constituents studied[a]							
	Quercetin-3-O-rutinoside (Rutin)	Quercetin-3-galactosides	Kaempferol-3-rutinoside	Quercetin-3-O-rhamnoside (Quercetrin)	Kaempferol-3-(*p*-coumaryl) glucoside	Quercetin	Isorhamnetin	Kaempferol
Raw Ginkgo leaves								
$Blank_{aqueous}$	0.190(1.38%)	0.033(2.96%)	0.109(0.34%)	0.015(0.39%)	N.D.	N.D.	N.D.	N.D.
$Blank_{alcohol}$	1.88(2.0%)	0.579(5.24%)	1.31(1.28%)	0.354(0.9%)	0.015(0.62%)	0. 0243(3.16%)	0.002(7.35%)	0.015(7.4%)
EGb 761								
$Blank_{aqueous}$	4.47(0.67%)	6.26(2.21%)	3.67(0.26%)	4.58(0.08%)	1.63(1.19%)	0. 212(5.77%)	0.027(1.97%)	N.D.
$Blank_{alcohol}$	9.28(3.22%)	4.60(3.73%)	1.67(7.1%)	0.738(5.81%)	1.60(0.98%)	0. 046(13.22%)	1.24(3.77%)	0.06(8.8%)
Commercial capsules								
$Blank_{aqueous}$	0.753(1.68%)	0.073(5.91%)	0.220(4.2%)	0.816(1.13%)	1.09(0.76%)	0. 131(2.85%)	N.D.	N.D.
$Blank_{alcohol}$	25.3(1.53%)	1.08(5.45%)	0.532(6.35%)	1.51(1.47%)	2.32(1. 3%)	11.0(2.61%)	1.31(6.94%)	3.64(3.4%)

N.D. = Not detected.
[a] Mean of 3 determinations (% RSD).

Table 3
Percentage recovery of the various compounds from raw *Ginkgo biloba* leaves following specific treatment

Sample code	Treatment	Quercetin-3-O-rutinoside (Rutin)	Quercetin-3-galactosides	Kaempferol-3-rutinoside	Quercetin-3-O-rhamnoside (Quercetrin)	Kaempferol-3-(*p*-coumaryl) glucoside	Quercetin	Isorhamnetin	Kaempferol
$A_{aqueous}$	Gastric 1 h	73.2	6347	231	55.5	NIL	NIL	NIL	NIL
$B_{alcohol}$	Gastric 1 h	7.75	376	22.8	3.65	NIL	NIL	NIL	NIL
$C_{aqueous}$	Gastric 1 h + Intestinal 2 h	116	122	1019	40.4	NIL	NIL	NIL	NIL
$D_{alcohol}$	Gastric 1 h + Intestinal 2 h	9.22	6.30	74.4	5.78	NIL	NIL	NIL	NIL
$E_{aqueous}$	Gastric 1 h + Intestinal 4 h	125	10901	441	154	NIL	NIL	NIL	NIL
$F_{alcohol}$	Gastric 1 h + Intestinal 4 h	9.79	501	29.3	4.94	NIL	NIL	NIL	NIL

Table 4
Percentage recovery of the various compounds from the EGb 761 following various treatments

Sample code	Treatment	Quercetin-3-O-rutinoside (Rutin)	Quercetin-3-galactosides	Kaempferol-3-rutinoside	Quercetin-3-O-rhamnoside (Quercetrin)	Kaempferol-3-(*p*-coumaryl) glucoside	Quercetin	Isorhamnetin	Kaempferol
$A_{aqueous}$	Gastric 1 h	85.1	13.6	119	4.55	9.70	78.4	NIL	NIL
$B_{alcohol}$	Gastric 1 h	41.0	18.8	860	43.8	18.2	391	NIL	54.9
$C_{aqueous}$	Gastric 1 h + Intestinal 2 h	88.6	17.3	176	6.38	17.7	90.3	NIL	NIL
$D_{alcohol}$	Gastric 1 h + Intestinal 2 h	46.3	25.2	422	43.1	18.7	416	NIL	25.9
$E_{aqueous}$	Gastric 1 h + Intestinal 4 h	98.5	18.5	190	7.74	20.3	106	NIL	NIL
$F_{alcohol}$	Gastric 1 h + Intestinal 4 h	40.1	22.3	382	30.6	2.89	79.5	NIL	NIL

Table 5
Percentage recovery of the various compounds from commercial Ginkgo capsules following specified treatments

Sample code	Treatment	Quercetin-3-O-rutinoside (Rutin)	Quercetin-3-galactosides	Kaempferol-3-rutinoside	Quercetin-3-O-rhamnoside (Quercetrin)	Kaempferol-3-(*p*-coumaryl) glucoside	Quercetin	Isorhamnetin	Kaempferol
$A_{aqueous}$	Gastric 1 h	7.42	341	22.0	2.00	1.60	9.03	NIL	NIL
$B_{alcohol}$	Gastric 1 h	0.135	124	4.72	0.864	0.294	0.055	NIL	0.055
$C_{aqueous}$	Gastric 1 h + Intestinal 2 h	7.54	39.7	0.813	1.21	0.389	0.000	NIL	NIL
$D_{alcohol}$	Gastric 1 h + Intestinal 2 h	0.207	3.17	39.4	0.000	0.909	0.110	NIL	NIL
$E_{aqueous}$	Gastric 1 h + Intestinal 4 h	6.28	43.3	96.7	1.20	0.222	0.000	NIL	NIL
$F_{alcohol}$	Gastric 1 h + Intestinal 4 h	0.220	3.45	36.5	0.810	0.621	0.118	NIL	NIL

However, when subjected to prolonged incubation under digestion conditions, even though the cellulose is not digested, there is likely to be cellular disruption and an increased release of flavonoid glycosides available for extraction, thus explaining the apparent increases (recovery of up to >1.25 times for rutin, >63 times for quercetin-3-galactosides, >2.3 times for kaempferol-3-rutinosides and >1.53 times for quercetin-3-O-rhamnosides) in the results obtained. The findings also seem to suggest low yields of flavonoids from undigested leaves because the compounds are chemically bound to the cell walls or within the intact cells and not available for extraction.

3.3. EGb 761

For EGb 761, reference to Table 2 indicates a similar trend in the distribution of glycosides and aglycones in the aqueous and alcoholic medium. Following the simulated digestion procedure, the quantification of the various flavonoids, in the aqueous extract of EGb761, differs (Table 4). The following percentages, compared with the undigested samples of the various flavonoids identified, were observed, ca. 80–90% for rutin, ca. 10–20% for quercetin-3-O-galactosides, ca. 100–200% for kaempferol-O-rutinosides, ca. 4–8% for quercetin-3-O-rhamnosides, ca. 9–20% for kaempferol-3-(*p*-coumaryl) glucoside and ca. 70–105% for quercetin. Isorhamnetin and kaempferol were not detected.

If the simulated digestion system does break down the glycosides, this should be reflected in an increase in aglycone recovery in the alcoholic medium after digestion. The breakdown of the glycosides is indicated by less than 100% recovery of rutin, quercetin-3-galactosides and quercetin-3-O-rhamnosides in the aqueous acidic extract. Differences in the glycoside/aglycone ratio, most likely reflect differences in stability of the glycosides.

Interestingly, kaempferol-3-rutinoside, which appears to be a more stable glycoside, does show an increased extraction following more severe digestion and hence is likely to be more available for potential absorption into the body. In contrast, kaempferol-3-(*p*-coumaryl) glucoside, shows a poor recovery in both the alcoholic and aqueous extract, indicating a likely total breakdown. However, following the 4th h of incubation, kaempferol-3-(p-coumaryl) glucoside seems to be degraded, indicating instability of the aglycone previously formed.

In sample D (alcohol) (Table 4), it may be noted that the sum of the recoveries for kaempferol-3-rutinoside and quercetin adds up to 838%. As the starting material in this experiment was an extract, even if all eight compounds tested had been recovered in their entirety, the maximum total figure is in theory only 800%. This apparent de novo production of flavonoids is most likely explained by the low yield of recovery of flavonoids from the undigested samples.

3.4. Commercial capsules

Similar to the results seen in the pervious samples, the undigested aglycones were better extracted in the alcoholic medium compared with the aqueous medium (Table 2). Isorhamnetin and kaempferol were not detected in the aqueous medium.

Following the simulated digestion (Table 5), the rutin recoveries were ca. 6% and 0.2%, respectively, in the aqueous and alcoholic extracts. However for quercetin-3-galactosides an increase of 34 times and 1.24 times, respectively, for aqueous and alcoholic extract occurred after the 1st h. After the full simulated digestion procedure, there was a reduction in the amounts to 30–40% and 3–3.5% recovery in the aqueous and alcoholic media, respectively. Recovery of quercetin-3-O-rhamnosides also gave a low percentage recovery (ca. 0–2% for the aqueous and alcoholic media). Such low recovery, seen in the quercetin glycosides, would be expected to be accompanied by a higher recovery in the corresponding aglycones. However, this was not observed. The recovery of quercetin was also low giving ca. 9% recovery in the aqueous medium after the 1st h of digestion and subsequently showing no recovery when subjected to the intestinal conditions. This seems to indicate that there was little or no conversion of glycosides to aglycones and, in addition, any aglycones so formed appear to have been degraded in the simulated digestion process.

As above, it appears that the kaempferol-3-rutinoside is relatively stable. However, the percentage recovery of kaempferol was almost insignificant following the simulated digestion, thereby indicating a similar phenomenon; i.e., there was little or no conversion of glycosides to aglycones and, in addition, it seems that the latter has been degraded in the simulated digestion process.

On the other hand, kaempferol-3-(*p*-coumaryl) glucoside, showed very low recovery (ca. 0–2% for the aqueous and alcoholic media). This indicated that kaempferol-3-(*p*-coumaryl) glucoside was fairly unstable and could have been degraded to yield non-active compounds.

3.5. Summary of effects

Degradation of the flavonoid compounds in gastrointestinal fluids has been reported to be minimal, at ca. 5%, in ileostomy subjects ($n = 6$) (Hollman et al., 1995). A similar finding is also observed in this work. In the digested *Ginkgo* leaves, a higher concentration of flavonoid glycosides than with the undigested leaves was seen. However, in contrast, this study seems to indicate that the recovery of selective flavonoid compounds in EGb 761 and commercial capsules, after incubation in vitro in simulated gastrointestinal fluids, is minimal. For quercetin-3-galactosides, quercetin-3-O-rhamnosides and kaempferol-3-(*p*-coumaryl) glucoside in EGb 761, and

for all the eight constituents of the Ginkgo capsules, a very low recovery was observed of only ca. 20%.

This observation seem to suggest caution when expressing the beneficial effect that these constituents may provide. From various reports, based on epidemiological research, a hypothesis has been proposed of an inverse relationship between the dietary intake of some flavonoids and the incidence of several chronic diseases (Hertog, Hollman, & Katan, 1992; Knekt et al., 2002). However, whether the hypothesis is true or not has still to be confirmed in view of the controversial subject of flavonoid absorption (Hollman & Katan, 1997; Hollman et al., 1997a; Hollman, van Trijp, Mengelers, de Vries, & Katan, 1997b). This paper has not addressed the absorption issue but merely the digestion aspect of the flavonoids.

Based on the above results, it appears that some of the active flavonoid compounds in the commercially prepared samples are produced in lower amounts after the simulated digestion procedure than after with the digestion of the natural leaves. This may be due to previous losses of these compounds during the preparation of the commercial samples. By taking in the raw leaves as opposed to the commercial preparations examined, there is likely to be a greater benefit in view of the higher level of flavonoids detected.

From Table 2 it may be seen that the undigested commercial extracts do in fact contain a higher proportion of aglycones than the raw Ginkgo leaves and thus, only when the controversy of glycoside vs aglycone absorption has been fully resolved, will it became clear which products are most likely to provide the greatest in vivo benefits.

4. Conclusion

The different *Ginkgo biloba* samples seem to have reacted differently to the simulated gastrointestinal fluid. The results indicate a trend of conversion from the glycosides to the aglycones and subsequent degradation for the commercial preparations. However, for the raw leaves, no conversion of glycosides to aglycones was observed. In addition, the digestion process appears to increase the availability of the glycosides compared with the undigested sample. For the EGb 761, the quercetin glycosides seem to have been converted to their aglycone, showing four times the amount recovered from the control while, for the commercial capsules, most of the constituents were not recovered, indicating degradation of the flavonoids constituents.

With the increase in popularity of dietary supplements as a source of flavonoids with health benefits, this result is significant as it indicates that the raw materials (in this case, the leaf of the *Ginkgo biloba* plant) is a more efficient medium for absorption of potentially beneficial flavonoids.

However, it is important to note that the commercial products seem to have a higher level of aglycones. Thus, an important area of further research is to establish the validity of the absorption of glycosides vs aglycones in the human body. While there is no doubt about the benefits of *Ginkgo biloba*, its therapeutic functions should now be considered in light of the data from this study, and, in particularly, which of the compounds within the plant are actually available and absorbed to bestow their physiological benefits.

However, care should be exercised in the interpretation of this study as no consideration was given to the possible interaction that may exist with other components present in the diet, e.g., proteins, lipids and minerals, etc. Also, this study did not investigate possible effects on flavonoids exerted by enzymes from the brush border membrane nor the microorganisms in the colon.

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