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Citation	European Journal of Nutrition, 59(4), 1389-1398 https://doi.org/10.1007/s00394-019-01992-9	
Issue Date	2019	
Doc URL	http://hdl.handle.net/2115/85218	
Rights	The final publication is available at link.springer.com	
Туре	article (author version)	
File Information	Final manuscript.pdf	



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11 Abstract

Purpose To investigate the effects of water-soluble dietary fibers (pectin, soybean fiber, and guar gum) on the bioavailability of quercetin glucoside mixture (Q3GM) comprising quercetin-3-*O*-glucoside (Q3G, 31.8%) and its glucose adducts.

Methods Male Wistar/ST rats were fed test diet containing 0.7% Q3GM with or without 5% of each dietary fiber for 8 weeks. Total quercetin derivatives were evaluated with liquid chromatograph tandem mass spectrometry (LC-MS/MS) as total quercetin derivatives after enzymatic deconjugation in plasma, urine, and fecal samples on week 2, 4, 6 and 8. Quercetin glucuronides excreted in feces were also measured.

20 **Results** Fiber feeding elevated cecal weight and reduced cecal pH, indicative of cecal 21 fermentation promotion. Changes in plasma and urinary quercetin levels revealed three phases 22 of quercetin metabolism, including cumulative, transient, and stable phases. On week 2, total 23 quercetin derivatives were higher in plasma samples from three fiber-fed groups than those 24 control groups; however, urinary excretion increased in fiber-fed groups on week 4. Soybean 25 fiber upregulated plasma and urinary quercetin levels on week 6 and 8. Intestinal degradation 26 of quercetin by bacteria, calculated from differences between aglycone ingestion and sum of 27 urinary and fecal excretion, was suppressed after dietary fiber supplementation especially in 28 pectin fiber, which may partly contribute to the increase in quercetin bioavailability. Fecal 29 quercetin glucuronide excretion was high in soybean fiber-fed rats, suggestive of the reduction 30 of β -glucuronidase in colon.

31 Conclusion Water-soluble dietary fibers, especially soybean fiber, enhanced quercetin
 32 bioavailability after chronic feeding and may promote beneficial effects of quercetin on disease
 33 prevention.

34

35 Keywords: Quercetin; Water-soluble dietary fiber; Bioavailability; Fermentation; Degradation

36 Introduction

37 Quercetin is an abundant natural flavonoid belonging to the class of flavonol. It is usually 38 present in fruits and vegetables in glycosylated forms (sugar-bound) such as quercetin-3- $O-\beta$ -39 d-glucoside (Q3G). Several studies have demonstrated the biological activities of quercetin on 40 prevention of diseases such as colon cancer, cardiovascular diseases, and diabetes [1-4]. The 41 bioavailability of quercetin and its glycosides is low, limiting their beneficial effects [5]. 42 Furthermore, the bioavailability of quercetin varies depending on coexisting food ingredients. 43 There are two mechanisms related to quercetin glucoside absorption in the intestine. In 44 the first mechanism, lactase-phlorizin hydrolase cleaves sugar moieties from quercetin 45 glucosides [6] and the resulting aglycone is absorbed into intestinal epithelial cells by simple 46 diffusion. In the second absorptive pathway, quercetin glucosides are transported via a glucose transporter [7-8] and the absorbed quercetins are conjugated as glucuronides and sulfates, or 47 48 methylated in the epithelial cells. Quercetin derivatives are further conjugated and methylated 49 in the liver. These quercetin conjugates in the blood are excreted into the urine and bile. 50 Excreted quercetin conjugates into the bile are reabsorbed in the intestine. Urinary excretion 51 under stable condition (after saturation with quercetin in the body) reflects the intestinal 52 absorption rate of quercetin [9]. The unabsorbed quercetin glucosides and aglycones in the 53 small intestine flow into the cecum and colon, wherein quercetin is absorbed into the 54 epithelium or broken down by the intestinal bacteria [10-12]. The degradation of quercetin 55 aglycone in the large intestine may be the reason for the low bioavailability of quercetin [13,14]. 56 Conjugated forms of quercetin excreted into the bile also reach into the large intestine, wherein 57 these molecules are usually degraded by the bacterial β -glucuronidase. The high activity of this enzyme is known to increase the risk of colon cancer [15,16]. 58

59 Dietary fibers are non-digestible polysaccharides that prevent the onset of several 60 metabolic diseases through physicochemical properties and intestinal fermentation. Being 61 resistant to the digestive enzymes in the small intestine, dietary fibers increase the fermentation 62 and production of organic acids, mainly short-chain fatty acids (SCFAs), in the large intestine 63 [17,18]. Clinical studies have demonstrated that the consumption of dietary fibers attenuates 64 severe increases in blood glucose, cholesterol, and triglyceride after a meal and lowers the risk of disease development [19,20]. In recent studies, the bioavailability of quercetin glycosides 65 was shown to be increased by co-ingested saccharides, including non-digestible 66 oligosaccharides, depending on the intestinal bacterial metabolism [21-23]. These studies 67 68 suggest that water-soluble dietary fibers fermented by intestinal bacteria promote 69 bioavailability of dietary flavonoids. However, the effects of chronic feeding of water-soluble 70 dietary fibers on quercetin bioavailability have not been studied.

In this study, we used pectin and guar gum as representative water-soluble and fermentable dietary fibers. We also included soybean fiber, a fermentable fiber with low viscosity. We hypothesized that the continuous ingestion of dietary fibers may increase quercetin bioavailability by promoting intestinal fermentation and that the viscous property of fibers affects the intestinal absorption of quercetin.

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77 Materials and methods

78 Chemicals

Quercetin-3-*O*-glucoside mixture (Q3GM, kindly supplied by San-Ei Gen F.F.I., Inc., Osaka, Japan) comprised quercetin-3-*O*-glucoside (Q3G, 31.8%) and mono (23.3%), di (20.3%), tri (9.7%), tetra (6.8%), penta (4.3%), hexa (2.6%) and hepta (1.1%) glucose adducts of Q3G with α -1,4-linkages, which was quantified by LC-MS/MS analyses described below by using individual standard compounds [23]. We used Q3GM because absorption of this quercetin glucosides is higher than Q3G (natural compound) [21] and already used as a food additive with granted generally recognized as safe (GRAS) status in Japan. Soybean fiber, a 86 highly fermentable fiber with low viscosity, was kindly donated by Fuji oil Co., Ltd (Osaka, 87 Japan). Pectin from citrus and guar gum were purchased from Wako Pure Chemical Industries, 88 Ltd (Osaka, Japan) and Tokyo chemical industry Co., Ltd (Tokyo, Japan), respectively. As 89 deconjugating enzymes, β -glucuronidase ($\geq 100,000$ units/mL) and sulfatase ($\geq 2,000$ 90 units/mL) from *Helix pomatia* extract (Cat. No. S9751) and sulfatase (10-20 units/mL) from 91 Aerobacter aerogenes extract (Cat. No. S1629) were purchased from Sigma Aldrich (Saint 92 Louis, USA). We used two sources of sulfatases because we found that insufficient hydrolysis 93 of some sulfate conjugates by Helix pometia enzyme. Other reagents were provided by Wako 94 unless specified.

95

96 Animals and diets

97 Male Wistar/ST rats, weighing about 120-140 g (5 weeks old) purchased from Japan SLC, 98 Inc. (Hamamatsu, Japan), were housed in an individual stainless-steel cage with wire-mesh 99 bottom. Rats were maintained in a controlled environment (12 h light/dark period, relative 100 humidity 40-60%, and temperature $22^{\circ}C \pm 2^{\circ}C$) with ad libitum access to diet and water. Rats 101 were assigned to five groups (n = 7 rats/group) after 1-week acclimation with a sucrose-based 102 diet based on AIN 93G formulation [24]. Five groups of rats were fed with different diets as 103 follows; sucrose-based AIN 93G diet for basal diet group (BA): control diet (sucrose-based 104 AIN 93G diet mixed with 0.7% Q3GM) for control group (Q): control diet mixed with 5% of 105 each test fiber which are pectin or soybean fiber or guar gum for QP, QS, and QG group, 106 respectively. In the diet component, sucrose is replaced by supplemented Q3GM and cellulose 107 is replaced by the test fibers for fiber treatment groups. The percentage (0.7%) of Q3GM used 108 in the present study was equivalent to 0.5% of quercetin aglycone, as our previous study [21]. 109 Body weight and food consumption of rats were measured daily. Tail vein blood was collected 110 at 10.00 - 11.30 a.m. on week 2, 4, 6 and 8 after feeding test diets. Urine and feces were

111 collected for 2 and 3 days, respectively, in the same week before blood collection day to 112 evaluate quercetin bioavailability. For urine collection, 0.05% sodium azide was added to 113 prevent degradation of quercetin metabolites by bacterial growth. Collected urine samples were 114 filled up to 100 mL, and 15 mL of samples were stored at -40°C. Fecal samples were stored at 115 -80° C. At the end of feeding period, rats were anesthetized with sodium pentobarbital injection 116 (Somnopentyl, Kyoritsu Seiyaku Corporation, Tokyo, Japan) and killed by withdrawal of the 117 aortic blood followed by exsanguination after overnight fasting. The liver and epididymal fat 118 pad were collected and weighed. The cecum and its contents were collected for measurements 119 of pH, SCFAs, and other organic acids.

This study was approved by the Hokkaido University Animal Committee, and the animals
were maintained in accordance with the Guide for the Care and Use of Laboratory Animals of
Hokkaido University.

123

124 Sample treatments

125 Plasma or diluted urine sample (100 μ L) was acidified with 10 μ L of 0.58 mol/L acetic 126 acid and treated with 7.5 μ L of each deconjugation enzyme, β -glucuronidase and sulfatase [23]. 127 Enzyme treated-sample was incubated at 35°C for 1 h and naringenin (2 µmol/L as an internal 128 standard) was added to all samples. The mixture was centrifuged $(9,300 \times g \text{ at } 4^{\circ}\text{C} \text{ for } 5 \text{ min})$ 129 and the supernatant was collected; the precipitate was re-extracted with methanol. The 130 supernatant was loaded onto a C18 cartridge (Oasis HLB, Waters Co. LTD, Milford, MA, 131 USA) and eluted with 1 mL methanol. After drying and reconstitution in a 50% methanol 132 solution, sum of quercetin and methylquercetin after treatment of deconjugation enzymes (total 133 quercetin derivatives) were measured by liquid chromatography tandem mass spectrometry 134 (LC-MS/MS).

135 Fecal samples were freeze dried and milled and their dry weights measured. Powdered 136 fecal samples (0.1 g) were suspended in 1 mL of 80% methanol solution and treated with 100 137 µL of 10 µmol/L naringenin as internal standard [23]. Mixtures were homogenized using 138 ultrasonic homogenizer (VP-050, Saitama, Japan) and incubated at 60°C for 1 h before 139 centrifugation $(2,300 \times g \text{ for } 10 \text{ min at } 4^{\circ}\text{C})$. Methanol (1 mL) was added to the precipitate and 140 homogenized with Teflon homogenizer (42W, ULTRAS homogenizer, Taitec Co. Ltd., 141 Nagoya, Japan). The mixture was centrifuged to precipitate insoluble materials, including 142 proteins. The entire procedure was repeated twice. The obtained supernatant was evaporated 143 and the concentrations of total quercetin derivatives were measured as the same as plasma and 144 urine extracts.

145

146 Quercetin measurement by LC-MS/MS

147 Total quercetin derivatives, sum of deconjugated quercetin and methylquercetin, were 148 determined using LC-MS/MS system [25] (TSQ Quantum Access Max with Accela High 149 Speed LC System, Thermo Fisher Scientific Inc., MA, USA) with an electrospray ionization 150 (ESI) source with SRM mode. The analytical column was a 1.9 µm C18 column (Inertsustain 151 Swift C18, 2.1 mm × 100 mm GL Sciences Inc., Tokyo, Japan) set at 40°C. The mobile phase 152 was water, methanol, and formic acid (70:30:0.1, solvent A), and methanol with formic acid 153 (100:0.1, solvent B). The ratio of solvent A and B was delivered through a linear gradient 154 (90:10 for 1 min; 90:10-20:80 for 8 min; 90:10 for 1 min) at a flow rate of 0.2 mL/min. 155 Concentrations of total quercetin derivatives were calculated from the calibration curve of 156 standard compounds.

157

158 Cecum treatment for pH and SCFAs measurement

159 Frozen cecum samples were thawed and cecal contents and tissues were separated and 160 individually weighed. Cecum contents were diluted with four times weight of deionized water, 161 and the pH value measured with a handy pH meter (B-211 twin waterproof pH, Horiba, Ltd., 162 Kyoto, Japan). Diluted samples (700 µL) were mixed with 200 µL of 25 mM crotonic acid 163 (internal standard) in 50 mM sodium hydroxide (NaOH), and centrifuged. The supernatants 164 were mixed with the same volume of chloroform and centrifuged again. The supernatant was filtrated through a 0.2 µm membrane, and concentrations of organic acids, including SCFAs, 165 166 were measured with high-performance liquid chromatography (HPLC) constructed with two 167 shim-pack SCR-102H (8 mm I.D. \times 300 mm L Shimadzu Corp.) and conductivity detector 168 CCD-6A (polarity: +, response: slow, temperature: 45°C; Shimadzu Corp.). The flow rate of 169 the mobile phase (5 mM aqueous *p*-toluenesulfonic acid) and buffer (5 mM *p*-toluenesulfonic 170 acid solution containing 100 µM ethylenediaminetetraacetic acid [EDTA] and 20 mM Bis-171 Tris) were 0.8 mL/min. The concentration of each organic acid was calculated from peak area 172 of chromatogram and the calibration curve of each standard organic acid corrected by the 173 internal standard.

174

175 Statistics

Data are presented as means with standard errors of the mean (SEM). Statistical significance was assessed using one-way analysis of variance (ANOVA) with Tukey-Kramer's test. Two-way ANOVA was carried out to verify the effect of the duration of feeding test (week i.e., weeks 2, 4, 6, and 8) and diet treatment (group i.e., Q, QP, QS, and QG) on values of plasma, urine, feces quercetin aglycone as well as quercetin aglycone degradation. P < 0.05was considered as significant. Statistic calculation was performed by JMP version 13.0 (SAS Institute Inc., Cary, NC).

184 <u>Results</u>

185 Food intake, body weight, and organ and dry fecal weights

Final body weight in QP group was lower than that reported in other groups, while daily food intake reduced following ingestion of dietary fibers without any significant difference (Table 1). Relative wet weight (grams per 100 g body weight) of the livers showed no change following dietary fiber treatment, but that of epididymal fat pad was lower in QS group than control group.

The weights of cecum tissues and cecum contents were significantly higher in QS and QG groups, but not in QP group, than basal and control groups. Maximum weight was reported for the samples from QS group (Table 2). Values of pH of the cecum contents were lower for samples from QP, QS, and QG groups than those from basal and control groups, indicative of the promotion of cecal fermentation by respective water-soluble fibers. Cecal pool of total organic acids, which is the sum of all acids shown in Fig. 1, and that of acetic and propionic acids were higher in QS group than other groups.

Dry weights of feces (Table 3) collected on week 2, 4, 6, and 8 showed lower values in QP, QS, and QG groups than basal and control groups, suggesting that dietary fibers added to diets were similarly degraded by intestinal bacterial fermentation.

201

202 Total quercetin derivatives in plasma

Total quercetin derivatives are shown in Fig. 2. Concentrations of methylquercetin derivatives were higher than those of quercetin derivatives. On week 2, feeding Q3GM group with individual water-soluble fibers elevated the concentrations of total quercetin derivatives. From 2 to 6 weeks, plasma total quercetin derivatives gradually decreased in all groups. On week 6 and 8, all groups of rats showed steady levels of total quercetin derivatives, with the highest value reported for QS group. Two-way ANOVA results revealed that both duration of feeding and diet treatment influenced the plasma concentrations of total quercetin derivatives and showed significant interaction (week × group: P = < 0.0001).

- 211
- 212 **Total quercetin derivatives in urine**

In the urine samples collected on week 2, the level of total quercetin derivatives was significantly higher in QG group than in control group. Both QS and QG groups had higher values than control group on week 4 (Fig. 3). From week 4 to 8, urinary levels of total quercetin gradually decreased in all groups. Urinary quercetin level in QS group was higher than that in all other groups on week 6 and 8. The fluctuation in total quercetin excretion was influenced by both feeding period and dietary treatment, as per two-way ANOVA results, and showed significant interactions (week × group: P = 0.0066).

220

221 **Total quercetin derivatives in feces**

222 Fecal excretion of total quercetin derivatives was higher in QP group than control group 223 through 8-week feeding period, while ingestion of soybean fiber (QS group) increased the 224 excretion of total quercetin from week 4 to 8 (Fig. 4). QG group showed increased tendency of 225 fecal total guercetin throughout the experiment. According to two-way ANOVA results, 226 dietary treatment, but not feeding period, influenced the fecal total quercetin derivatives. As 227 shown in Fig. 5, quercetin glucuronides in feces were almost undetectable in control (Q) group, but were detectable in QP and QS groups. In particular, total glucuronide levels (quercetin 228 229 glucuronides and methylquercetin glucuronides) gradually increased in QS group and were 230 higher than the other groups.

231

232 Degradation of quercetin and methylquercetin aglycones

233 We found that the amounts of quercetin derivatives excreted in urine and feces samples 234 were lower than the levels of ingested quercetin, suggestive of the degradation of large amounts 235 of quercetin aglycones by intestinal bacteria (Table 4). The amount of quercetin and 236 methylquercetin aglycone degraded was calculated by subtracting sum of aglycone excreted 237 into urine and feces from total aglycone ingested. The differences between ingested and 238 degraded aglycone is remaining aglycone survived from degradation by intestinal bacteria. The 239 percentages of remaining aglycone was approximately two times higher in water-soluble fiber 240 groups than the control group, and the values in QP group were the highest throughout the 241 experiment (approximately five times higher than the control group). The degradation ratio 242 was lower in all fiber groups than control group and the lowest value was reported for QS 243 group. These differences were maintained during the 8-week feeding period, which was 244 supported by the absence of any significant interaction between period and dietary treatment 245 as analyzed with two-way ANOVA (P = 0.8832).

246

247 Discussion

248 We observed the effects of three water-soluble dietary fibers on the bioavailability of a 249 quercetin glucoside in rats in an 8-week feeding study by monitoring the changes in the 250 concentrations of quercetin derivatives in the plasma, urine, and feces samples. The results of 251 plasma and urine quercetin levels suggest three phases in quercetin metabolism, including 252 cumulative (~week 2), transient (week 4), and stable (week 6-8) phases. The daily intake of 253 quercetin glucoside with individual water-soluble fibers for 2 weeks increased the plasma 254 concentrations of total quercetin derivatives, suggestive of the enhanced intestinal absorption 255 of the ingested quercetin. However, urinary excretion of total quercetin derivatives, which 256 usually reflects the bioavailability of ingested flavonoids, failed to increase in QP and QS 257 groups. On week 2, quercetin absorption may have increased after water-soluble fiber feeding 258 and the absorbed quercetin accumulated in the blood and tissues in these groups. Mullen et al. 259 have reported that accumulation of radio-labelled quercetin in tissues [26]. In contrast, urinary 260 excretion of quercetin derivatives in QG-fed rats largely increased on week 2. Quercetin in the 261 body was probably saturated in QS group earlier than other fiber groups due to higher absorption of quercetin. The greater effect of guar gum for absorption of quercetin glycosides 262 263 is not known, however, we speculate fermentation of guar gum is faster than pectin and soybean 264 fiber on week 2. On week 4, urinary excretion of quercetin in OP, OS, and OG groups tended 265 to be or was largely increased, but the increased plasma quercetin concentrations disappeared 266 on week 6 except in QS group. These results suggest that week 4 is a transient phase of 267 increased total quercetin derivatives. From week 6 to 8, levels of plasma quercetin and urinary 268 excretion reduced but stabilized, with significantly higher levels observed in QS group than 269 BA group. On week 6 of all groups, the reduction of quercetin derivatives in both plasma and 270 urinary levels suggests the reduction of quercetin bioavailability that possibly be associated 271 with the increasing degradation of quercetin aglycone. However, we found increase in fecal 272 excretion of quercetin from week 4 to week 6 in QS group. We speculated that the reduction 273 of quercetin in plasma and urinary excretion on week 6 is associated with suppression of 274 intestinal absorption of quercetin by prolonged feeding of Q3GM by a feedback mechanism.

275 In recently, some intestinal bacteria catabolize are able to convert quercetin aglycone to 276 phenolic acids such as 3-(3,4-dihydroxyphenyl) propionic acid and 3,4-dihydroxy-277 phenylacetic acid through ring scission process [27,28]. These degraded products are known 278 to have strong anti-oxidative properties and also effective for prevention of many diseases, 279 which depends on anti-oxidative ability [29,30]. However, quercetin has more various actions 280 independent of anti-oxidative properties, for example strong interaction with cell-signaling 281 proteins [31]. Some researches have been reported that phenolic acids possessed a low ability 282 to suppress the carcinogenesis compared with quercetin aglycone [32,33]. However, adaptation 283 of intestinal bacteria to long-term feeding of quercetin may increase the levels of the enzymes 284 that decompose quercetin aglycone mainly in the cecum [34,35]. In this experiment, we 285 observed an increase in the fecal excretion of quercetin derivatives following pectin (QP) and 286 soybean fiber (QS) diet feeding, observed probably through the suppression of quercetin 287 degradation. We calculated the remaining aglycone survived from bacterial degradation as 288 shown in Table 5. The amount of remaining aglycone, the restoration rates of quercetin 289 aglycone through the avoidance of bacteria degradation in rats, largely increased in QP and QS 290 groups on week 6 and 8. A possible explanation for this observation is that dietary fibers were 291 used as energy source instead of quercetin by cecal bacteria [36,37]. *Bacteroidetes* spp. are 292 known to upregulate the expression of several genes related to carbohydrate-metabolizing 293 enzymes and may use ingested fibers as substrate [38]. The suppression of bacterial 294 degradation of quercetin aglycone may be responsible for the constant high levels of quercetin 295 in plasma and urinary excretion in QS group. In QP group, no increase in quercetin levels in 296 plasma and urine was observed for the maximum amount of available aglycone. The high 297 viscosity property of pectin in the intestinal lumen possibly suppressed quercetin absorption in 298 the small intestine, and this property might also contribute to the low bioavailability in guar 299 gum-fed rats during the late phases.

300 Supplementation with fibers, especially soybean fiber, largely and gradually increased the 301 fecal excretion of quercetin and methylquercetin glucuronides. This finding indicates that 302 soybean fiber suppressed the deconjugation of quercetin glucuronides. Cermak et al. [39] 303 reported the transformation of the conjugated metabolites of quercetin to aglycone by certain 304 strains of *Pediococcus* spp., *Streptococcus* spp., *Lactobacillus* spp., *Bifidobacterium* spp., and 305 *Bacteroides* spp. Rowland et al. [40] also found a significant increase in β -glucuronidase 306 activity in rats fed with 5% pectin diet, consistent with the results of the present study that 307 glucuronide levels in feces reduced from week 4 to 6 and 8 in pectin group (QP). In QS group,

fecal glucuronide levels were higher than those in other groups and further increased after longterm dietary fiber ingestion. Bacterial β -glucuronidase activity in the large intestine is one of the strong inducers of colon cancer [41]. Ingested soybean fiber may suppress the proliferation of β -glucuronidase-producing bacteria or inhibit β -glucuronidase activity in the large intestine. Therefore, feeding soybean fiber may serve as a strategy to prevent colon cancer.

313 In the present study, we hypothesized that ideal dietary fibers may enhance quercetin 314 bioavailability by increasing cecal fermentation and suppressing quercetin degradation. Pectin 315 showed the highest ability to suppress bacterial degradation of quercetin aglycone and seemed 316 to be the best candidate for enhancing blood levels of quercetin, but it was true only in the early 317 phase of feeding (week 2). We revealed that soybean fiber is the most effective fiber to enhance 318 quercetin bioavailability and blood levels among three water-soluble and fermentable dietary 319 fibers for a long-term feeding studied herein. Soybean fiber is known to increase mucosal 320 surface area with the expansion of cecal wall [42], consistent with the increase in cecal contents 321 and tissue weight observed in the present study. Soybean fiber has relatively low viscosity as 322 compared with pectin and guar gum [43,44] that may have contributed to the prolonged effects 323 on quercetin bioavailability.

In conclusion, we found that water-soluble dietary fibers, especially soybean fiber, enhanced quercetin glycoside bioavailability following long-term feeding of rats. Duration of feeding is an important factor to modulate these abilities of dietary fibers. The findings of the present study suggest that dietary fibers promote the actions of quercetin on prevention of diseases.

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468	Fig. 1 Pools of organic acids in cecal contents of rats fed a sucrose-based AIN 93G (BA) diet
469	with 0.7 % Q3GM (Q) with 5% pectin (QP) or soybean fiber (QS) or guar gum (QG). Total

470 organic acids (sum of succinic, lactic, acetic, propionic, iso-butyric and n-butyric acids). Values 471 are mean \pm SEM (n = 7) and not sharing a common letter differ significantly (P = < 0.05) by 472 Tukey-Kramer's post hoc test. All *P*-values of one-way ANOVA were <0.0002 for each of 473 organic acid and total organic acids except 0.1664 for succinic acid.

474

Fig. 2 Plasma concentration of quercetin and methyl quercetin derivatives in the tail blood of rats fed the sucrose-based AIN 93G diet with 0.7 % Q3GM (Q) with 5% pectin (QP) or soybean fiber (QS) or guar gum (QG). Values are mean \pm SEM (n = 7) and not sharing a common letter differ significantly (P < 0.05) by Tukey-Kramer's post hoc test.

480 **Fig. 3** Urinary excretion of quercetin and methyl quercetin derivatives of rats fed the sucrose-

 $481 \qquad based \ AIN \ 93G \ diet \ with \ 0.7 \ \% \ Q3GM \ (Q) \ with \ 5\% \ pectin \ (QP) \ or \ soybean \ fiber \ (QS) \ or \ guar$

482 gum (QG). Values are mean \pm SEM (n = 7) and not sharing a common letter differ significantly

483 (P < 0.05) by Tukey-Kramer's post hoc test.

484

485Fig. 4 Fecal excretion of quercetin and methyl quercetin derivatives of rats fed the sucrose-486based AIN 93G diet with 0.7 % Q3GM (Q) with 5% pectin (QP) or soybean fiber (QS) or guar487gum (QG). Values are mean \pm SEM (n = 7) and not sharing a common letter differ significantly488(P < 0.05) by Tukey-Kramer's post hoc test.</td>

489

490 Fig. 5 Fecal excretion of quercetin and methyl quercetin monoglucuronide metabolites of rats

491 fed the sucrose-based AIN 93G diet with 0.7 % Q3GM (Q) with 5% pectin (QP) or soybean

492 fiber (QS) or guar gum (QG). Values are mean \pm SEM (n = 7) and not sharing a common letter

493 differ significantly (P < 0.05) by Tukey-Kramer's post hoc test.



Fig.1







Fig.4



Group	Initial BW (g.)	Final BW (g.)	Food intake (g/day)	Liver (g/100 g BW)	Epididymal fat pad (g/100 g BW)
BA	162 ± 3	426 ± 11	19.46 ± 0.58	0.79±0.02	1.51±0.16 ^ª
Q	162 ± 2	432 ± 14	19.53 ± 0.66	0.80 ± 0.01	1.31 ± 0.03^{ab}
QP	162 ± 2	405 ± 15	18.31 ± 0.38	0.71±0.01	1.34 ± 0.07^{ab}
QS	162 ± 2	425 ± 13	18.52 ± 0.56	0.73±0.02	1.12 ± 0.07^{b}
QG	162 ± 2	432 ± 14	18.87 ± 0.53	0.78 ± 0.02	1.34 ± 0.05^{ab}

Table 1 Initial body weights, final body weight, average food intake, liver and epidydimal fad pad weight of rats fed a sucrose-based AIN 93G diet (BA) with 0.7 % Q3GM (Q) with 5% pectin (QP) or soybean fiber (QS) or guar gum (QG).

Values are mean \pm SEM (n = 7) and not sharing a common letter differ significantly (*P* < 0.05) by Tukey-Kramer's post hoc test. *P* values for one-way ANOVA were 0.998 for initial body weight, 0.3802 for final body weight, 0.3417 for average food intake, 0.5201 for liver weight and 0.0396 for epididymal fat pad.

Table 2 Whole cecum weight, cecum tissue weight, content weight and pH value of the cecal's rats fed a sucrose-based AIN 93G diet (BA) with 0.7 % Q3GM (Q) with 5% pectin (QP) or soybean fiber (QS) or guar gum (QG).

Group	Whole cecum weight (g/100 g BW)	Cecum tissue weight (g/100 g BW)	Content weight (g/100 g BW)	рН
BA	$0.77 \pm 0.01^{\circ}$	0.23 ± 0.01^{d}	$0.54 \pm 0.07^{\circ}$	6.7 ± 0.1^{a}
Q	$0.82 \pm 0.04^{\circ}$	$0.27 \pm 0.01^{\circ}$	$0.55 \pm 0.05^{\circ}$	6.6 ± 0.1^{a}
QP	0.97 ± 0.07^{bc}	0.30 ± 0.01^{bc}	$0.67 \pm 0.12^{\circ}$	6.1 ± 0.0^{b}
QS	3.35 ± 0.45^{a}	0.41 ± 0.01^{a}	2.94 ± 0.25^{a}	6.1 ± 0.1^{b}
QG	1.88 ± 0.15^{b}	0.35 ± 0.02^{b}	1.53 ± 0.12^{b}	6.1 ± 0.0^{b}

Values are mean \pm SEM (n = 7) and not sharing a common letter differ significantly (*P* < 0.05) by Tukey-Kramer's post hoc test. All *P*-values of one-way ANOVA were <0.0001.

Group -	Total dry fecal weight (g/3days)			
ereap -	Week 2	Week 4	Week 6	Week 8
BA	5.22 ± 0.36 ^ª	5.46 ± 0.35^{a}	5.38 ± 0.25^{a}	5.51 ± 0.15 [°]
Q	4.93 ± 0.19^{a}	5.49 ± 0.30^{a}	5.64 ± 0.29^{a}	5.87 ± 0.24^{a}
QP	2.59 ± 0.26 ^b	3.23 ± 0.16^{b}	3.10 ± 0.24^{b}	3.53 ± 0.20^{b}
QS	2.00 ± 0.18 ^b	2.28 ± 0.15 ^b	3.31 ± 0.20^{b}	3.02 ± 0.19^{b}
QG	2.23 ± 0.14	3.23 ± 0.28^{b}	3.11 ± 0.39^{b}	3.42 ± 0.29^{b}

Table 3 Total dry fecal weight of rats fed a sucrose-based AIN 93G diet (BA) with 0.7 % Q3GM (Q) with 5% pectin (QP) or water-soluble soybean fiber (QW) or guar gum (QG).

Values are mean \pm SEM (n = 7) and not sharing a common letter differ significantly (P < 0.05) by Tukey-Kramer's post hoc test. All *P*-values of one-way ANOVA were <0.05.

Group	Ingested aglycone (µmol/day)	Excreted aglycone* (µmol/day)	Remaining aglycone** (%)	Degraded aglycone*** (%)
Week2				
Q	195.91 ± 5.44 ^ª	6.69 ± 1.67^{b}	3.42 ± 0.85^{b}	96.58 ± 0.85^{a}
QP	177.42 ± 7.46^{b}	33.65 ± 4.97^{a}	18.97 ± 2.82 ^ª	81.03 ± 2.82^{b}
QS	171.72±5.07 ^b	11.37 ± 2.64^{b}	6.65 ± 1.60^{b}	93.35±1.60 ^ª
QG	169.73±3.60 ^b	12.77 ± 1.66 ^b	7.59 ± 1.07^{b}	92.41±1.07 ^ª
<u>Week4</u>				
Q	202.77 ± 7.75	$6.22 \pm 0.99^{\circ}$	$3.12 \pm 0.53^{\circ}$	96.88 ± 0.53^{a}
QP	187.39±4.57	32.76 ± 3.43^{a}	17.63±1.98 ^ª	82.37±1.98 [°]
QS	188.23±5.54	17.10 ± 2.72^{b}	9.20±1.57 ^b	90.80±1.57 ^b
QG	189.62±4.74	13.59 ± 2.88^{bc}	7.13±1.52 ^{bc}	92.87±1.52 ^{bc}
<u>Week6</u>				
Q	206.87 ± 7.51	$6.23 \pm 1.20^{\circ}$	$2.97 \pm 0.53^{\circ}$	97.03 ± 0.53^{a}
QP	193.36±5.18	26.14 ± 3.59^{a}	13.52±1.72 ^ª	86.48±1.72 [°]
QS	193.75±5.71	19.40 ± 1.30^{ab}	10.08±0.78 ^{ab}	89.92 ± 0.78^{bc}
QG	197.56±5.22	11.38 ± 2.99^{bc}	5.84 ± 1.57 ^{bc}	94.16±1.57 ^{ab}
Week8				
Q	209.19±7.54	5.40 ± 1.02^{b}	2.55 ± 0.43^{b}	97.45 ± 0.43^{a}
QP	195.79±4.80	34.28 ± 5.91^{a}	17.33 ± 2.86^{a}	82.67 ± 2.86 ^b
QS	197.60 ± 6.05	17.75±2.29 ^b	9.13±1.31 ^b	90.87±1.31 ^b
QG	201.56±5.02	10.03 ± 3.45^{b}	4.98 ± 1.71^{b}	95.02±1.71 ^b
Two-way ANOVA				
Week	<0.0001	0.8679	<0.0001	0.6599
Group	0.0004	<0.0001	<0.0001	<0.0001
Week ×	0.9299	0.4457	0.8832	0.4099
<u>Week6</u> Q QP QS QG <u>Week8</u> Q QS QG wo-way AN Week Group Week × Group	206.87 ± 7.51 193.36 ± 5.18 193.75 ± 5.71 197.56 ± 5.22 209.19 ± 7.54 195.79 ± 4.80 197.60 ± 6.05 201.56 ± 5.02 OVA <0.0001 0.0004 0.9299	$6.23 \pm 1.20^{\circ}$ 26.14 ± 3.59^{a} 19.40 ± 1.30^{ab} 11.38 ± 2.99^{bc} 5.40 ± 1.02^{b} 34.28 ± 5.91^{a} 17.75 ± 2.29^{b} 10.03 ± 3.45^{b} 0.8679 <0.0001 0.4457	2.97 ± 0.53^{c} 13.52 ± 1.72^{a} 10.08 ± 0.78^{ab} 5.84 ± 1.57^{bc} 2.55 ± 0.43^{b} 17.33 ± 2.86^{a} 9.13 ± 1.31^{b} 4.98 ± 1.71^{b} <0.0001 <0.0001 0.8832	97.03 ± 0.53^{a} 86.48 ± 1.72^{c} 89.92 ± 0.78^{bc} 94.16 ± 1.57^{ab} 97.45 ± 0.43^{a} 82.67 ± 2.86^{b} 90.87 ± 1.31^{b} 95.02 ± 1.71^{b} 0.6599 < 0.0001 0.4099

Table 4 Amount of quercetin aglycone degraded per day and rate (%) of remaining aglycone in rats fed test diet containing a sucrose-based AIN 93G diet with 0.7 % Q3GM (Q) with 5% pectin (QP) or soybean fiber (QS) or guar gum (QG).

Values are mean \pm SEM (n = 7) and not sharing a common letter differ significantly (P = < 0.05) by Tukey-Kramer's post hoc test.

* Excreted aglycone (μ mol/day) = sum of total aglycone excreted into urine and feces

** Remaining aglycone (%) = 100 - (degraded aglycone/ingested aglycone x 100)
*** Degraded aglycone (%) = degraded aglycone/ingested aglycone x 100