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Original Scientific paper

Degradation kinetics of fisetin and quercetin in solutions affected by medium pH, temperature and co-existing proteins

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Abstract: The impacts of medium pH, temperature and co-existing proteins on the degradation of two flavonoids, fisetin and quercetin, were assessed in the present study using a spectroscopic method. Based on the measured degradation rate constants (k), fisetin was more stable than quercetin in all cases. Increasing the medium pH from 6.0 to 7.5 at 37 °C enhanced the respective k values of fisetin and quercetin from 8.30×10^{-3} and 2.81×10^{-2} to 0.202 and 0.375 h^{-1}, respectively ($p < 0.05$). In comparison with their degradation at 37 °C, fisetin and quercetin showed larger k values at higher temperature (0.124 and 0.245 h^{-1} at 50 °C, or 0.490 and 1.42 h^{-1} at 65 °C). Four protein products in the medium at 0.10 g L^{-1} stabilized the two flavonoids ($p < 0.05$), as they decreased the respective k values of fisetin and quercetin to 2.28×10^{-2}––2.98×10^{-2} and 4.37×10^{-2}–5.97×10^{-2} h^{-1}. Hydrophobic interactions between the proteins and the two flavonoids were evidenced responsible for the stabilization, as sodium dodecyl sulfate could destroy the stabilization significantly ($p < 0.05$). Casein and soybean protein provided greater stabilization than whey protein isolate. It was thus concluded that higher temperature and alkaline pH could enhance flavonoid loss, whereas the coexistence of proteins as flavonoid stabilizers could inhibit flavonoid degradation.

Keywords: fisetin; quercetin; degradation kinetics; temperature; pH; proteins.

INTRODUCTION

Flavonoids are polyphenolic compounds characterized with a C6–C3–C6 backbone[1] and are categorized into mainly six groups, *i.e.*, flavones, flavonols, flavanols, flavanones, flavanonols and isoflavones. Flavonoids are usually abundant in plant-based foods, such as onions, apples, berries, tea and red wine, and belong to the most important components of the well-known phytochemicals.

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Many researchers have focused their attention on the health benefits of flavonoids to the body, for example, antioxidant and especially anti-cancer properties.[2] Flavonoids such as myricetin, quercetin, and rutin can protect DNA from damage in both Caco-2 and Hep G2 cells induced by H_2O_2,[3] while those from cocoa are able to prevent high glucose-induced oxidative stress on HepG2 cells.[4] The anticancer properties of flavonoids are among the most studied topics, for example, flavones and flavonols show *in vitro* cytotoxicity to human oesophageal adenocarcinoma cell line (OE33) and human oesophageal squamous cell carcinoma cell line (KYSE-510).[5,6] Overall, the potential health benefits of flavonoids have been widely studied and clarified. However, most of the previous studies essentially focused on the *in vitro* bioactivity of the flavonoids. Whether food processing and co-existing food components (*e.g.*, proteins, carbohydrates and others) have impacts on the bioactivity and stability of flavonoids have not hitherto been well studied.

Flavonoids contain sensitive chemical groups and structural elements in their molecules and are thus susceptible to degradation accelerated by many factors. The chemical instability of flavonoids mainly arises from the hydroxyl groups and the instable pyrone structure (*i.e.*, the second ring).[7,8] It was observed that the degree of hydroxylation of flavones and flavonols exerts a significant impact on their stability, in the decreasing order of resorcinol-, catechol- and pyrogallol-types. However, glycosylation of these hydroxyl groups obviously resulted in enhanced stability.[8] It was also found that flavonoids can interact with some food components, especially with macromolecular materials.[1] Interaction between proteins and polyphenols affect the taste of tea and coffee, antioxidant properties, and protein digestibility.[9] Complexation of tea polyphenols with milk proteins can change the antioxidant activity of the polyphenols and the secondary structure of the proteins.[10] Interaction of starch with tannins and other phenolic compounds was found adverse to starch digestibility.[11] Moreover, increasing the protein level of the milk decreased the degradation of the flavonoid compound epigallocatechin gallate at two temperatures.[12] These results indicate the importance of co-existing compounds or components on flavonoid stability.

Plant foods usually undergo necessary thermal processing and storage, during which flavonoids degradation occurs. The processing conditions and co-existing compounds or components in the plant foods might affect the degradation of flavonoids and, consequentially, the bioactivity of the flavonoids. These factors should therefore be assessed. Strawberry and onion are rich in the two flavonoids fisetin and quercetin.[13] In the present study, their degradation kinetics in solutions were investigated *via* a spectroscopic method. The impacts of medium pH, temperature and some widely used protein ingredients on their degradation were assessed. The aim of the present study was to reveal the chemical stability of fisetin and quercetin and their stabilization by proteins.

EXPERIMENTAL

Materials and chemicals

Fisetin and quercetin (purity > 98 %) were purchased from the Shanghai Yousi Biotechnology Co. Ltd. (Shanghai, China) and the Dalian Meilun Biotechnology Co. Ltd. (Dalian, China), respectively. Dimethyl sulfoxide (DMSO) was obtained from the Solarbio Science and Technology Co. Ltd. (Beijing, China). Casein (protein content of 88.93 % on dry basis), whey protein isolate (WPI) (protein content of 87.95 % on dry basis) and defatted soybean flour were purchased from the Beijing Aoboxing Bio-Tech Co. Ltd. (Beijing, China), Brewster Dairy (Brewster, OH, USA), and the Harbin Hi-tech Soybean Food Co. Ltd. (Harbin, Heilongjiang, China), respectively. All other employed chemicals were of analytical grade.

Preparation of soybean protein samples

Defatted soybean flour was extracted at ambient temperature with 75 vol. % ethanol by stirring the suspension for 12 h, using a flour-to-solvent ratio of 1:10 (*w*/*V*). After the extraction, the precipitate was collected and re-extracted again using the same ethanol solution until the separated ethanol solution no longer showed a yellow color upon addition of a NaOH solution (100 mmol L^{-1}). The obtained precipitate (*i.e.* defatted and dephenolized soybean flour) was dried at ambient temperature overnight, and used to prepare soybean the protein isolate (SPI) according to the method of Petruccelli and Añón.[14] The SPI was then dephenolized (assigned as DSPI), which was adjusted to pH 7.0, and freeze-dried to obtain a powder sample. A DSPI solution prior to freeze-drying was heated at 85 °C for 20 min, cooled, freeze-dried to obtain thermally denatured DSPI (assigned as DDSPI).

The protein contents of the protein samples were determined by the classic Kjeldahl method,[15] using a conversion factor of 6.25.

Spectroscopic analysis of fisetin and quercetin solutions during storage

Both fisetin and quercetin were dissolved in DMSO to obtain a stock solution of 300 mmol L^{-1}, and then stored at 4 °C before use. The stock solutions were diluted to 3 mmol L^{-1} with ethanol, and then further diluted using a phosphate buffer (100 mmol L^{-1}, pH 6.8) to 30 μmol L^{-1}. The two diluted solutions were incubated in a water bath of 37 °C. After incubation times of 1–6 h, their spectra (200–600 nm) were recorded on a UV–Vis spectrophotometer (UV-2401 PC, Shimadzu Co. Kyoto, Japan), using the buffer as blank.

Assay of the degradation kinetics of fisetin and quercetin at different medium temperatures and pH values

The stock fisetin and quercetin solutions were diluted to 3 mmol L^{-1} with ethanol, and then diluted by three phosphate buffers (100 mmol L^{-1}, pH 6.0, 6.8 and 7.5), respectively, to a concentration near 30 μmol L^{-1}. The diluted solutions of pH 6.8 were incubated in a water bath at three respective temperatures (37, 50 and 65 °C). The diluted solutions of pH 6.0 and 7.5 were incubated in a water bath at 37 °C. Absorbance at 360 and 368 nm of the solutions were measured at different incubation time intervals, to detect residual fisetin and quercetin concentrations, respectively, using a UV–Vis spectrophotometer and the respective buffers as blanks. The fisetin and quercetin concentrations were calculated based on the standard curves generated prior to the measurements.

To ensure efficient degradation and precise spectroscopic analysis, the incubation time intervals used for fisetin and quercetin solutions were as follows: at pH 6.8, the fisetin solutions were measured at every 60 (37 °C), 30 (50 °C) and 12 (65 °C) min, while the quer-

cetin solutions were measured at every 30 (37 °C), 15 (50 °C), and 6 (65 °C) min. At pH 6.0 and 7.5, the fisetin solutions were measured at every 120 and 30 min, while the quercetin solutions were measured at every 60 and 30 min.

Assay of the stabilization of fisetin and quercetin by co-existing proteins

Four protein samples, including casein, DSPI, DDSPI and WPI, were all dispersed in phosphate buffer (100 mmol L^{-1}, pH 6.8). The stock fisetin and quercetin solutions were also diluted as above using ethanol and the buffer, but merged with the prepared protein solutions, to give final fisetin and quercetin concentrations of ≈30 μmol L^{-1} as well as protein concentrations of 0.05–0.20 (casein) and 0.10 (other proteins) g L^{-1}. Subsequently, the generated solutions were kept in a water bath at 37 °C, and the residual fisetin and quercetin concentrations were measured as above. The time intervals used for fisetin and quercetin solutions were 60 and 30 min, respectively. The quantitative analyses of fisetin and quercetin were also based on the generated standard curves.

Statistical analyses and calculation of the degradation rate constants

All experiments and analyses were performed in triplicate. Data were analyzed by SPSS 16.0 software (SPSS, Inc., Chicago, IL, USA), and are expressed as means ± standard deviations. Based on the first-order reaction model,[16] degradation rate constants (k) of fisetin and quercetin were thus calculated using the derived linear regression equation:

$$\ln c_t = A - kt \quad (1)$$

where c_t is the detected concentration (μmol L^{-1}) of fisetin or quercetin in the solution treated for different times; t is treatment time (h) of the solution and k is degradation rate constant (h^{-1}); A is a constant.

RESULTS AND DISCUSSION

Kinetics of the degradation of fisetin and quercetin, as well as the effects of pH and temperature

The absorption spectra of flavonoids usually consist of two distinctive bands in a broad range of 240–400 nm: band I (300–380 nm) with maximum around 350–370 nm and band II (240–280 nm) with maximum around 260–270 nm. Both fisetin and quercetin exhibited chemical instability in the solutions, reflected by the obtained spectra scanned at 200–600 nm (Fig. 1). The spectra of fisetin and quercetin (with maximums at 360 and 368 nm, respectively), showed decreased absorption intensities in the two regions, behaving in a time-dependent manner (Fig. 1A and B, respectively), when kept at 37 °C for longer times. No new absorption peak was clearly observed in the measured wavelength range. Decreased absorption intensities of fisetin and quercetin in solutions evidenced the degradation of fisetin and quercetin, and long-time incubation resulted in greater degradation of fisetin and quercetin.

The influences of two medium factors (pH and temperature) on the degradation of fisetin and quercetin are shown in Fig. 2, which demonstrates that the concentrations of fisetin and quercetin decreased in a time-dependent manner. By using the first-order reaction model, the k values for fisetin and quercetin were calculated, and are listed in Table I. Some results were obtained based on the changes

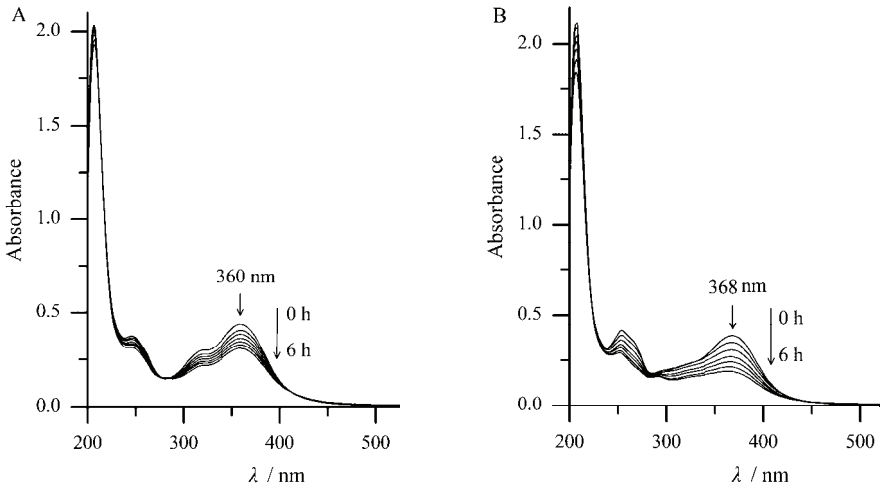

Fig. 1. The absorption spectra of fisetin (A) and quercetin (B) solutions (30 μmol L^{-1}, in 100 mmol L^{-1} phosphate buffer, pH 6.8) kept at 37 °C for 0–6 h.

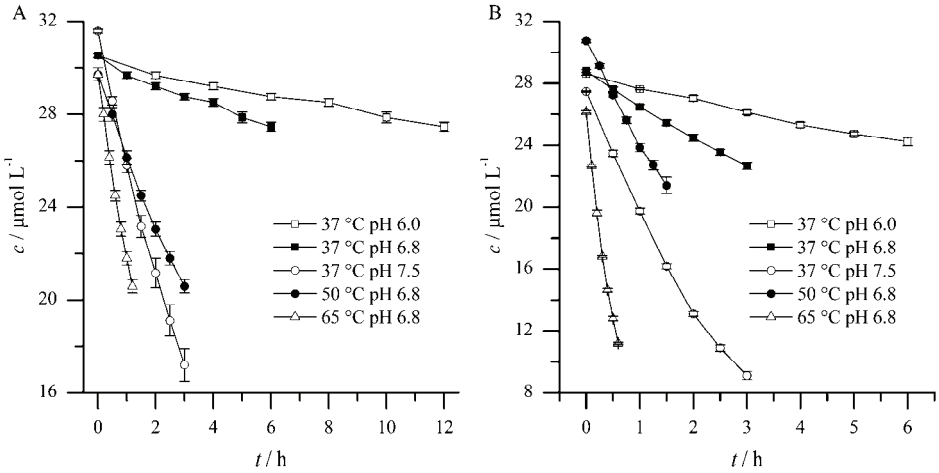

Fig. 2. Detected residual concentrations of fisetin (A) and quercetin (B) in solutions subjected to different temperatures, pH values and times.

in the *k* values under different pH values and temperatures. The first is that fisetin was more stable than quercetin, giving smaller *k* values in all cases. The second is that the degradations of fisetin and quercetin were sensitive to medium pH, especially at alkaline pH values. Increasing the medium pH value from 6.0 to 7.5 increased the *k* values of fisetin and quercetin by 24- and 12-fold (from 8.30×10^{-3} to 0.202 h^{-1} and from 2.81×10^{-2} to 0.375 h^{-1}), respectively. On the contrary, increasing the medium pH value from 6.0 to 6.8 enhanced the *k* values of fisetin and quercetin to 3.58×10^{-2} and 7.99×10^{-2} h^{-1}, respectively, *i.e.*, only a 3- and

2-fold increase, respectively. The third is that higher temperature resulted in greater degradation of fisetin and quercetin, as the *k* values for fisetin and quercetin were larger at higher temperatures. For example, if fisetin and quercetin were kept at a temperature higher than 37 °C (*i.e.* 50 or 65 °C), the measured *k* value of fisetin was enhanced to 0.124 or 0.490 h^{-1}, while that of quercetin was enhanced to 0.245 or 1.42 h^{-1}.

TABLE I. Impacts of medium temperature and pH value on the degradation rate constants (*k*) of fisetin and quercetin in solutions; different lowercase letters after the mean values as the superscripts in same column indicate that one-way ANOVA of the mean values was significantly different ($p < 0.05$). The coefficient (R^2) for the regression analysis of the rate constants ranged from 0.984 to 0.999

Medium conditions		k / h^{-1}	
Temperature, °C	pH values	Fisetin	Quercetin
37	6.0	$(8.30\pm0.45)\times10^{-3a}$	$(2.81\pm0.14)\times10^{-2a}$
37	6.8	$(3.58\pm0.11)\times10^{-2b}$	$(7.99\pm0.32)\times10^{-2b}$
37	7.5	0.202 ± 0.014^{c}	0.375 ± 0.008^{c}
50	6.8	0.124 ± 0.002^{d}	0.245 ± 0.011^{d}
65	6.8	0.490 ± 0.009^{e}	1.42 ± 0.10^{e}

Flavonoids in aqueous solutions show instability, resulting in concentration loss (*i.e.*, degradation). The degradation of flavonoids was clarified as a first-order reaction,[16] and the main degradation reactions involve oxidation, hydroxylation and ring-cleavage.[17–19] During the degradation of flavonoids (*e.g.*, quercetin), some degradation products, such as 2,4,6-trihydroxymandelate and 2,4,6--trihydroxyphenylglyoxylate, were proposed as intermediate products.[20] However, it was also suggested that the end products (polyhydroxybenzoic acids) undergo further degradation with the formation of phenolic carboxylic acids of lower molecular weights.[20] The degradation of flavonoids therefore gives complex product profiles. As flavonoids have larger family members, the degradation of flavonoids and the impacts of medium conditions still require detailed investigation in the future.

The stability of flavonoids depends on their chemical structure. For example, more hydroxyl groups in a flavonoid molecule leads to lower stability.[8] Fisetin and quercetin have 4 and 5 hydroxyl groups, respectively. Thus, it is reasonable that quercetin was more liable than fisetin. The medium pH value has an important influence on the degradation of flavonoids. A previous study reported that plant phenolic compounds were susceptible to pH change.[21] When assessing the thermal degradation of quercetin and rutin at 100 °C in solutions from weakly acidic pH 5.0 to alkaline pH 8.0, it was found that the two flavonoids were more unstable at alkaline pH.[7] Kırca *et al.* studied the effect of pH on the thermal stability of black carrot anthocyanins in solutions of six pH values (2.5–7.0), and observed that the stability of anthocyanin decreased at pH values larger than

5.0.[16] When the degradation of cyaniding 3-rutinoside was evaluated at 78 °C in citrate buffers of pH 2.5, 3.5 and 4.5, the measured *k* values were 1.44×10^{-5}, 2.58×10^{-5} and 2.80×10^{-5} s^{-1}, respectively,[22] *i.e.*, higher pH value brought about faster cyaniding 3-rutinoside degradation. These mentioned studies shared similar conclusion to the present data, supporting that fisetin and quercetin were more stable (but instable) under acidic (and alkaline) conditions.

Chemical reactions are accelerated by higher temperatures. When black carrot was heated at fixed pH 6.0, the *k* values of anthocyanins in the temperature range of 70–90 °C ranged from 4.15×10^{-2} to 0.138 h^{-1}.[16] When roselle anthocyanin in solution was heated at five temperatures (60, 70, 80, 90 and 100 °C), the measured *k* values were 0.6×10^{-3}, 1.0×10^{-3}, 1.6×10^{-3}, 3.6×10^{-3} and 7.9×10^{-3} min^{-1}, respectively.[23] De Paepe *et al*. studied the thermal treatment of apple juice samples over a temperature range of 80–145 °C, and observed that the degradation of 39 phenolic compounds increased with temperature rise.[24] It is also reasonable that higher temperature (50 or 65 °C) would result in fisetin and quercetin with larger *k* values.

Stabilization of fisetin and quercetin by co-existing proteins

When studying the degradation of fisetin and quercetin in solutions of pH 6.8 at 37 °C, some proteins were added into the medium to clarify their effects on the degradation of fisetin and quercetin. Based on the measured data (Fig. 3), the *k* values of fisetin and quercetin were calculated (Table II). These proteins were found to inhibit effectively the degradation of fisetin and quercetin ($p < 0.05$), as

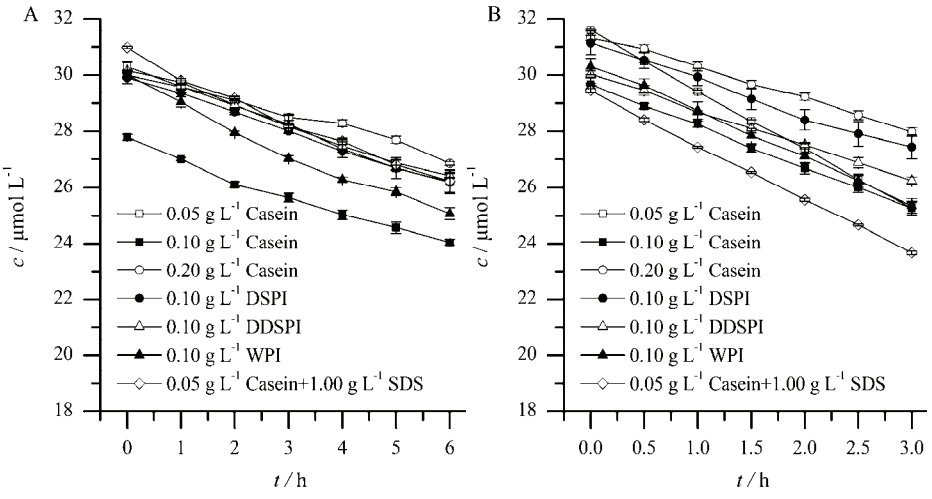

Fig. 3. The detected residual concentrations of fisetin (A) and quercetin (B) in the solutions kept at 37 °C, pH 6.8 for different times, in the presence of the assessed co-existing components. DSPI, DDSPI, WPI and SDS denote dephenolized soybean protein isolate, thermal denatured DSPI, whey protein isolate, and sodium dodecyl sulfate, respectively.

the measured *k* values decreased in all cases. The *k* values of fisetin were decreased from 3.58×10^{-2} to 1.76×10^{-2}–2.98×10^{-2} h^{-1}, *i.e.*, by about 17–51 %. The measured *k* values of quercetin decreased from 7.99×10^{-2} to 3.80×10^{-2}–5.97×10^{-2} h^{-1}, *i.e.*, by about 25–52 %. These results reflect that these co-existing proteins provided stabilization towards fisetin and quercetin in solution (*i.e.*, these proteins could act as flavonoid stabilizers). It is speculated that potential interactions between these co-existing proteins and the two flavonoids were responsible for the observed stabilization.

TABLE II. Impacts of the co-existing components on the degradation rate constants (*k*) of fisetin and quercetin in solutions. Different lowercase letters after the mean values as superscripts in same column indicate that one-way ANOVA of the mean values was significantly different ($p < 0.05$). The coefficient (R^2) if the regression analysis of the rate constants ranged from 0.979 to 0.999

Coexisting components	Levels, g L^{-1}	$k\times10^2$ / h^{-1}	
		Fisetin	Quercetin
None	0	$(3.58\pm0.11)^a$	$(7.99\pm0.32)^a$
Casein	0.05	$(2.49\pm0.20)^b$	$(7.42\pm0.20)^b$
Casein	0.10	$(2.37\pm0.06)^b$	$(5.36\pm0.29)^c$
Casein	0.20	$(1.76\pm0.02)^a$	$(3.80\pm0.10)^d$
DSPI	0.10	$(2.28\pm0.13)^b$	$(4.37\pm0.16)^e$
DDSPI	0.10	$(2.32\pm0.15)^b$	$(4.49\pm0.11)^e$
WPI	0.10	$(2.98\pm0.03)^c$	$(5.97\pm0.03)^f$
Casein + SDS	0.10 + 1.00	$(2.73\pm0.10)^d$	$(7.21\pm0.33)^b$

Some interesting results can be obtained from the data in Table II. It is seen that casein at 0.20 g L^{-1} provided the greatest stabilization towards fisetin and quercetin, as the measured *k* values of fisetin and quercetin were the lowest (1.76×10^{-2} and 3.80×10^{-2} h^{-1}). Decreased casein level brought about less stabilization towards fisetin and quercetin, as the measured *k* values of fisetin and quercetin showed decreasing trends. Another milk protein product WPI (at 0.10 g L^{-1}) showed much weaker stabilization of fisetin and quercetin than casein, as the measured *k* values of fisetin and quercetin were higher. Compared with casein, DSPI and DDSPI at 0.10 g L^{-1} could provide equal stabilization of fisetin (*k* values 2.28×10^{-2} *vs.* 2.32×10^{-2} h^{-1}), but to some extent stronger stabilization of quercetin (*k* values 4.37×10^{-2}–4.49×10^{-2} *vs.* 5.36×10^{-2} h^{-1}). Moreover, thermally denatured soybean protein had an unclear impact on the mentioned stabilization, because DSPI and DDSPI resulted in similar *k* values for fisetin and quercetin. However, the addition of SDS (an anionic detergent) to the medium clearly and effectively diminished the stabilization of fisetin and quercetin by casein ($p < 0.05$), as it led to enhancement of the respective *k* values of fisetin and quercetin from 2.37×10^{-2} to 2.73×10^{-2} h^{-1} and from 5.36×10^{-2} to 7.21×10^{-2}

h^{-1}. This fact indicates that SDS efficiently destroyed the interactions between the co-existing casein and the two flavonoids.

Past studies demonstrated that polyphenols can bind to proteins.[9,10] Song *et al*. reported that green tea flavan-3-ols could complex with milk proteins to enhance their stability.[12] Shpigelman *et al*. used native and thermally treated *β*-lactoglobulin to protect tea polyphenols, and found decreased degradation.[25] Xiao and Högger also observed that myricetin had a longer half period of life in human plasma than in a cell culture medium, and suggested that the higher total protein concentration (61.7 *vs*. 0.41 g L^{-1}) brought about greater myricetin stability.[8] The four protein products assessed in the present study were also capable of decreasing the degradation of fisetin and quercetin, especially casein and SPI. The present finding shared the same conclusion as those of these past studies, evidencing that these widely used protein products, acting as flavonoid stabilizers, could provide beneficial effect on the stability of fisetin and quercetin. Literature data show that casein and soybean protein contain more hydrophobic amino acids than whey protein.[26] Casein and soybean protein thus could interact with the two flavonoids much stronger than WPI, *via* well-known hydrophobic interactions. Consequentially, casein and soybean protein at the same protein concentration could provide stronger stabilization than WPI. However, hydrophobic interactions can be efficiently destroyed by detergents, *e.g*., SDS. If SDS was added into the fisetin and quercetin solutions together with casein, the hydrophobic interactions between the two flavonoids and casein were destroyed partly or mostly by SDS. Therefore, fisetin and quercetin were mostly in their free state and therefore degraded rapidly. It is thus concluded that hydrophobic interactions mostly contributed to casein stabilization of the two flavonoids. Flavonoids and proteins have hydrophilic (*e.g.* hydroxyl) groups in their molecules, which can also lead to the formation of hydrogen bonds between flavonoids and proteins. However, stabilization of the proteins towards flavonoids *via* hydrogen binding requires quantitative assaying in the future.

CONCLUSIONS

The stability and degradation kinetics of two flavonoids (fisetin and quercetin) in solution were impacted by medium conditions such as pH, temperature, and co-existing proteins. Quercetin with more hydroxyl groups was more instable than fisetin. Fisetin and quercetin were more sensitive at alkaline pH values and higher temperatures, resulting in larger degradation rate constants. However, co-existing proteins in the medium could provide stabilization of the two flavonoids, mainly *via* hydrophobic interactions between the proteins and the two flavonoids, resulting in fisetin and quercetin with decreased degradation rate constants. Casein and soybean protein products were better flavonoid stabilizers than whey protein isolate.

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ИЗВОД

УТИЦАЈ pH СРЕДИНЕ, ТЕМПЕРАТУРЕ И ПРИСУТНИХ ПРОТЕИНА НА КИНЕТИКУ РАЗЛАГАЊА ФИЗЕТИНА И КВЕРЦЕТИНА У РАСТВОРУ

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Утицај pH средине, температуре и присутних протеина на кинетику разлагања флавоноида физетина и кверцетина је испитиван спектрофотометријски. На основу одређених константи брзине деградације (k), закључено је да је физетин стабилнији од кверцетина у свим експерименталним условима. Повећањем pH средине са 6,0 на 7,5 на 37 °C расту k вредности за физетин и кверцетин од $8{,}30\times10^{-3}$ и $2{,}81\times10^{-2}$ до 0,202 и 0,375 h^{-1} ($p < 0{,}05$). У односу на брзину деградације на 37 °C, добијене су веће вредности k за физетин и кверцетин на већим температурама (0,124 и 0,245 h^{-1} на 50 °C, односно 0,490 и 1,420 h^{-1} на 65 °C). Четири протеинска производа у медијуму су могла стабилизовати ова два флавоноида ($p < 0{,}05$), пошто су при концентрацији од 0,10 g L^{-1} смањивали k вредности за физетин и кверцетин на $2{,}28\times10^{-2}$–$2{,}98\times10^{-2}$ и $4{,}37\times10^{-2}$–$5{,}97\times10^{-2}$ h^{-1}. Хидрофобне интеракције између протеина и флавоноида су заслужне за ову стабилизацију и ефекат стабилизације се губио у присуству натријум-додецил-сулфата ($p < 0{,}05$). Казеин и протеин из соје су обезбеђивали већу стабилност него протеин изолован из суротке. На основу свега је закључено да више температуре и алкалнији pH подстичу разградњу флавоноида, док их присуство протеина стабилизује.

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