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Uptake of phenolic compounds from plant foods in human intestinal Caco-2 cells

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In continuation of our studies on the bioaccessibility of phenolic compounds from food grains as influenced by domestic processing, we examined the uptake of phenolics from native/sprouted finger millet (*Eleucine coracana*) and green gram (*Vigna radiata*) and native/heat-processed onion (*Allium cepa*) in human Caco-2 cells. Absorption of pure phenolic compounds, as well as the uptake of phenolic compounds from finger millet, green gram, and onion, was investigated in Caco-2 monolayer model. Transport of individual phenolic compounds from apical compartment to the basolateral compartment across Caco-2 monolayer was also investigated. Sprouting enhanced the uptake of syringic acid from both these grains. Open-pan boiling reduced the uptake of quercetin from the onion. Among pure phenolic compounds, syringic acid was maximally absorbed, while the flavonoid isovitexin was least absorbed. Apparent permeability coefficient $P_{(app)}$ of phenolic compounds from their standard solutions was 2.02×10^{-6} cm/s to 8.94×10^{-6} cm/s. Sprouting of grains enhanced the uptake of syringic acid by the Caco-2 cells. Open-pan boiling drastically reduced the uptake of quercetin from the onion. The permeability of phenolic acids across Caco-2 monolayer was higher than those of flavonoids.

Keywords. Caco-2 cells; finger millet; green gram; onion; phenolic compounds; uptake

1. Introduction

Polyphenols are documented to exhibit many beneficial biological properties including antiinflammatory and antiatherosclerotic effects because of their antioxidant potential as well as their influence on related gene expression (Zhang 2015; Calabriso *et al*. 2016). Phenolic acids and flavonoids are the major classes of polyphenols distributed in plants. Cereal grains and vegetables are good source of these biologically active molecules. Finger millet (*Eleusine coracana*) and green gram (*Vigna radiata*), the common food grains of Asian and African populations (Devi *et al*. 2014; Kaur *et al*. 2015), supply significant amounts of phenolic compounds, while onion (*Allium cepa*), a common vegetable, is a major source of flavonoid in the diet (Galdón *et al*. 2008).

The concept of bioavailability of polyphenols needs to be understood in the context of their immense health beneficial physiological effects. Although cereal grains, legumes and vegetables are good sources of bioactive phenolic compounds, it is important that these compounds must be released from the food matrix and become bioavailable in order to achieve the specific activity. Bioaccessibility of phenolics from common cereal grains (finger millet, pearl millet, sorghum and wheat), legumes (green gram and chickpea) and a vegetable (onion) (Hithamani and Srinivasan 2014a, b, 2016a, b), as influenced by domestic processing, has been recently evaluated. It is observed that sprouting enhanced the bioaccessibility of phenolic compounds from cereal grains and legumes. Syringic acid was found to be the major bioaccessible phenolic compound from both finger millet as well as green gram, especially on sprouting. Isovitexin and quercetin are the common flavonoids of green gram and onion respectively (Cao *et al*. 2011; Henagan *et al*. 2014). Although ferulic acid and protocatechuic acid were not bioaccessible in finger millet, the same were found to be more bioaccessible from green gram on sprouting. Hence, it was proposed to study the differences in the uptake of these compounds using Caco-2 cell line as a model of the intestinal barrier. Although there are various reports on the natural sources of antioxidants, cereal grains being one among them, information on the bioavailability of

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these phenolic antioxidants from food grains and vegetables is scarce (Adam *et al*. 2002; Lee *et al*. 2014; Chitindingu *et al*. 2015).

Studies on the bioavailability of phenolic compounds from finger millet, green gram and onion are important in view of their immense health beneficial aspects attributable to polyphenols. Hypoglycemic, hypocholesterolemic, nephroprotective and anti-cataractogenic properties of seed coat matter (rich in phenolic compounds) of finger millet have been documented earlier (Shobana *et al*. 2010). Mung bean extract has shown effective inhibition on the formation of advanced glycation end products *in vitro* (Peng *et al*. 2008). Onion shows beneficial antioxidant and hepato-protective effect and thereby attenuated cholesterol gallstone formation in lithogenic diet–fed mice (Reddy and Srinivasan 2011).

Caco-2 cell line assay system, also known as 'golden standard' of intestinal cell models (Hubatsch *et al*. 2007), is an excellent model for studying transepithelial transport. The use of Caco-2 cell model for drug absorption measurements has been approved by the FDA Biopharmaceutical Classification system. Along with the feature of intestinal-like permeability, fully differentiated Caco-2 cell lines also exhibit active influx and efflux transporters (Na^+/H^+ antiporters) and metabolic enzymes such as UDP-glucuronosyl transferases and sulfotransferases (Sun *et al*. 2002; Seithel *et al*. 2006; Meinl *et al*. 2008). Previous bioavailability studies on polyphenols carried out using Caco-2 cell lines (Tenore *et al*. 2015; Willenberg *et al*. 2015) are mainly focused on the flavonoids, although phenolic acids are the most abundant forms of polyphenols found in nature. A recent study on the bioaccessibility and cellular uptake of polyphenol and carotenoid reports the employment of Caco-2 cells (Kaulmann *et al*. 2016).

The present investigation focuses on the uptake of phenolic acids (protocatechuic acid, syringic acid and ferulic acid) and flavonoids (isovitexin and quercetin) by the human Caco-2 cells. An attempt has also been made to study the bioavailability of these phenolic compounds, using Caco-2 cell model, from finger millet, green gram and onion. These representative cereal, pulses and vegetable were selected based on comparatively higher phenolic content; these also form the main constituents of common Indian composite meals.

2. Materials and methods

2.1 *Materials*

Finger millet (*Eleusine coracana*) and green gram (*Vigna radiata*) were procured from the National Seeds Corporation (Mysore, Karnataka, India). Onion (*Allium cepa*, dark red variety) was purchased from the local market in Mysore, Karnataka. Standard phenolic compounds, trifluoroacetic acid, pepsin, pancreatin and bile extract of porcine origin were procured from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Dulbecco modified Eagle's medium (DMEM), fetal bovine serum and other cell culture components were obtained from Himedia, India. Dimethyl sulfoxide (DMSO), MTT reagent [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] were of molecular biology grade. HPLC grade solvents were procured from Qualigens Chem. Co. (Mumbai, India). All other chemicals and reagents used in the experiments were of analytical grade.

2.2 *Sample processing*

Finger millet and green gram (10 g each in 30 mL water) were sprouted by soaking the grains overnight. Water was decanted, and grains were allowed to germinate under ambient conditions (25°C) for 48 h. Shade-dried germinated grains were powdered and was used for further analysis. Open-pan boiling of onion was carried out by boiling onion paste (10 g) in triple distilled water (20 mL) in an open-pan for 10 min at 85 ± 5°C. Native and processed grain/onion samples (10 g) were subjected to gastric and intestinal digestion (Hithamani and Srinivasan 2014a) as follows: Gastric digestion was initiated by incubation with pepsin (pH 2.0) at 37°C for 2 h. pH of the samples after gastric digestion was increased to 5 by adding 1 M sodium bicarbonate and pancreatin–bile extract was added. Intestinal digestion was carried out by incubating samples at 37°C with shaking for 2 h or longer until the pH of the digest reached 7.0. Samples were filtered through Whatman No. 1 filter paper, and the filtrate was used for further studies.

2.3 *Caco-2 cell culture*

Caco-2 cells were a kind gift from National Centre for Cell Science (Pune, India). Cells were cultured between passages 41–51 in a humidified atmosphere of 5% CO_2 and 95% air at 37°C in DMEM, supplemented with 10% heat-inactivated fetal bovine serum, 1% MEM non-essential amino acids and antibiotic-antimycotic solution. Media was replaced every alternate day and subcultured at 70–80% confluency (supplementary figure 1).

2.4 *Effect of phenolic compounds on the cellular viability*

Caco-2 cells were seeded in 96-well plates at a density of 10^4 cells/well. After 24 h of incubation, media was replaced, and cells were treated with serial concentrations (50, 100 and 500 μM) of standard phenolic compounds (protocatechuic

acid, syringic acid, ferulic acid, isovitexin and quercetin) in 200 µL serum-free media. Cells were also treated with digesta of native as well as processed finger millet, green gram and onion. Sodium dodecyl sulphate (SDS) and phosphate buffered saline (PBS) served as positive and negative controls in the experiment. Blank wells were also maintained to minus the background readings if any in the assay. At the end of the treatment period of 4 h, 20 µL (10 mg/mL) of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was added. After 3 h of incubation at 37°C, the media containing MTT was removed completely, and the reduced formazan dye was solubilized by DMSO (100 µL/well). The plate was kept in a plate shaker at 280 rpm for 10 min. Absorbance was measured at 570 nm using a spectrophotometric microtiter plate reader (Bio-Rad, USA). Results were expressed as the percentage of viable cells with 100% representing the control cells treated with only PBS.

2.5 *Phenolic compounds uptake*

Cells ($1 \times 10^4/cm^2$) were seeded in Corning Costar 6-well plate. The medium was changed on alternate days, and the experiments were performed after the cells attained 90–100% confluency. On the day of experiment, media was aspirated and cells were washed with phosphate buffered saline (pH 7.4). Individual standard phenolic compound or the food sample (the concentration was based on the phenolic content fixed as per MTT assay) dissolved in the media was loaded and incubated at 37°C for different time intervals (0, 1, 2, 3 and 4 h). Cells were washed with cold PBS twice. Cells were scraped into cytosol buffer (pH 7.5, 10 mM-Tris-HCl, 1 mM EDTA, 1 mM $MgCl_2$) maintained at 0–4°C, homogenized in a probe sonicator (IKA T10 basic Ultra-Turrax, USA) and centrifuged at 22000*g* for 25 min at 4°C. Supernatant (500 µL) was acidified with methanol (300 µL, 40% MeOH concentration) and used for HPLC analysis. The protein content of cell lysate was determined by Lowry's assay (Lowry *et al*. 1951). The concentration of food-derived phenolic compounds within the cells at different time intervals was calculated based on the concentration of standard phenolic compounds.

2.6 *Phenolic compounds transport*

Cells (1×10^5 per cm^2) were seeded in Corning Costar 6-well polycarbonate transwell plate inserts with an insert membrane pore size of 0.4 µm and a growth area of 4.67 cm^2. The experiments were performed 21 or 24 days post-seeding to obtain differentiated monolayer and transport of compound from apical to the basolateral chamber, across the monolayer was studied. The integrity of the monolayer was confirmed by incubating cells with Lucifer yellow (100 µM) for 4 h and subsequent measurement of fluorescence of the medium from the basolateral chamber, at an excitation wavelength of 427 nm and an emission wavelength of 535 nm in a microplate reader. On the day of the experiment, media was removed from the lower chamber followed by the upper chamber. Hank's balanced salt solution (HBSS) was added to both upper chamber (1.5 mL) and the lower chamber (2.6 mL) and incubated for 15 min at 37°C. Hank's balanced salt solution was then aspirated from the apical chamber and replaced with a fresh solution containing known concentration of standard phenolic compound (as per MTT assay result) and incubated at 37°C. Cells with only HBSS served as blank. Basolateral solution (1 mL) was removed at different time intervals (0, 1, 2, 3 and 4 h) and replaced with fresh HBSS. Immediately, the samples were mixed with methanol (200 µL) and centrifuged at 500*g* for 15 min. The supernatant sample was dried under vacuum and stored at −20°C until HPLC analysis.

Apparent permeability coefficient (P_{app}) value was calculated based on the transportation rate of each compound at different time intervals (Lee *et al.* 2014). The apparent permeability coefficient (P_{app}) of phenolic compounds expressed as cm/s was calculated according to the equation:

$$P_{app} = dc/dt \times V/(A \times C_0)$$

where dc/dt is the change in concentration in the receiving compartment over time, V is the volume of the solution in the receiving compartment (mL), A is the surface area of the membrane (cm^2), and C_0 is the initial concentration in the donor compartment (µM).

2.7 *HPLC analysis of phenolic compounds*

HPLC analysis was carried out using C18 analytical column (250 × 4.6 mm; 5 µm, maintained at 30°C, Model: Zorbax Eclipse Plus, Agilent Technologies Inc., Santa Clara, CA, USA) for all samples, which were cleaned-up by filtering through 0.20 µm Whatman filter before injection. The analysis was carried out in an Agilent HPLC system (1200 Series; Agilent Technologies Inc., Santa Clara, CA, USA). The mobile phase consisted of 0.1% trifluoroacetic acid (solvent A) and 100% methanol (solvent B). The flow rate was maintained at 1.0 mL/min for a total run time of 60 min with the gradient programme as follows: 20% B to 40% B in 40 min which was maintained for 10 min and then again to 20% B in next 5 min. There was 5 min of post-run for reconditioning. Sample (20 µL) was injected, and peaks were recorded simultaneously at 280 and 320 nm for the benzoic acid and cinnamic acid derivatives, respectively. Identification of the compounds was made based on the retention time of individual standard phenolic compounds

and concentration of compounds found in the sample was calculated with respect to the particular standard. Analysis of polyphenols from onion was carried out as follows: Solvent system used was water with acetic acid (pH 2.8)-solvent A and acetonitrile-solvent B. The gradient program followed was 0% to 10% B in 5 min, 10% to 23% B in 31 min, and 23% to 35% B in 43 min, followed by the column wash with 100% B for 6 min and equilibration for 6 min with 100% A before the injection of next sample. Injection volume was 20 μL, and the flow rate was 1.0 mL/min.

2.8 *Statistical analysis*

All determinations were made in three replicates, and the mean values are reported. Statistical analysis was carried out using GraphPad INSTAT, Version 3.06, GraphPad Software. Data were analysed by applying the one-way analysis of variance (ANOVA), and the differences between means were determined by Dunnet's test at a significance level of $P < 0.05$. Results for Apparent Permeability Coefficients of phenolic compounds in Caco-2 cells were analysed and the significance level was calculated using the Tukey–Kramer multiple comparison test.

3. Results

3.1 *Optimization of concentrations of phenolic compounds and food digesta*

The optimum concentration of individual phenolic standards (protocatechuic acid, ferulic acid, syringic acid, quercetin, isovitexin, and quercetin dihydrate) and digesta of the food sample (finger millet, green gram and onion) were determined by MTT assay. Percentage viability of Caco-2 cells at 50, 100 and 500 μM concentrations of standard phenolic compounds are shown in figure 1. Protocatechuic acid and isovitexin at 500 and 100 μM concentrations were found to be toxic to the cells. Similarly, 500 μM concentrations of other phenolic standards studied were toxic for the cells. Hence, protocatechuic acid and isovitexin at 50 μM concentration and syringic acid, ferulic acid, and quercetin at 100 μM concentration were employed in further studies. The optimum concentration of polyphenols obtained from the gastrointestinal digestion of native as well as processed finger millet, green gram and onion was also determined by MTT assay. The viability of Caco-2 cells at different concentrations of these samples are shown in figure 2. Strong inhibition of the viability of human Caco-2 cells was observed up to 0.01 μg/μL in the case of finger millet and green gram, and sprouted green gram at 0.05 μg/μL. There was no significant difference in the effect of native and sprouted finger millet on the viability of Caco-2 cells at all the concentrations studied. The percent viability of Caco-2 cells in the presence of digesta of the native green gram was low (68%) when compared to sprouted grain (117%) at 0.05 μg/μL. Onion showed even stronger inhibition to Caco-2 cells up to 0.001 μg/μL concentration. However, inhibition was more in the case of native onion when compared to open-pan boiled onion. Hence, the concentration of finger millet and green gram was fixed at 0.01 μg/μL, whereas for onion it was fixed at 0.001 μg/μL for further studies.

3.2 *Uptake of phenolic compounds by intestinal Caco-2 cells*

Optimized concentrations of phenolic compounds were added to the cells and incubated for 1, 2, 3 and 4 h. Uptake of different phenolic compounds by the Caco-2 cells is shown in figure 3. Each phenolic compound showed differential absorption at different time intervals. Percent uptake of phenolic compounds by the Caco-2 cells ranged from 0% to 21% (supplementary figure 2). The highest absorbed phenolic compound by Caco-2 cells was syringic acid, while the lowest was isovitexin which is a flavonoid. Uptake of protocatechuic acid by Caco-2 cells was minimal accounting to only 0.62 μM/mg protein at the end of 3 h out of 50 μM initial concentration, which however increased to 1.11 μM/mg protein at 4 h. Uptake of syringic acid, which gradually increased to 21.49 μM/mg protein by 3 h, was however decreased to 15.83 μM/mg protein at 4 h. A similar trend was observed in the case of ferulic acid, where it reached a maximum absorption of 4.72 μM/mg protein at 3 h. However, absorption of quercetin increased at 4 h.

Uptake of syringic acid by Caco-2 cells from digested native as well as sprouted finger millet at different time

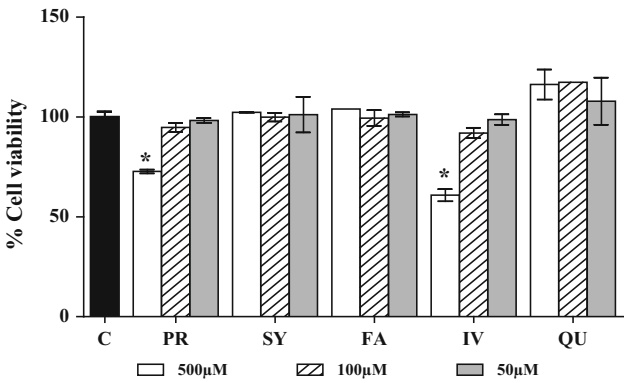

Figure 1. Viability of Caco-2 cells after 4 h of incubation with phenolic compounds dissolved in serum-free medium at different concentrations (50, 100 and 500 μM) (PR: protocatechuic acid; SY: syringic acid; FA: ferulic acid; IV: isovitexin, QU: quercetin). Values are mean ± SD of quadruplicates. *Significantly different from control ($P < 0.05$).

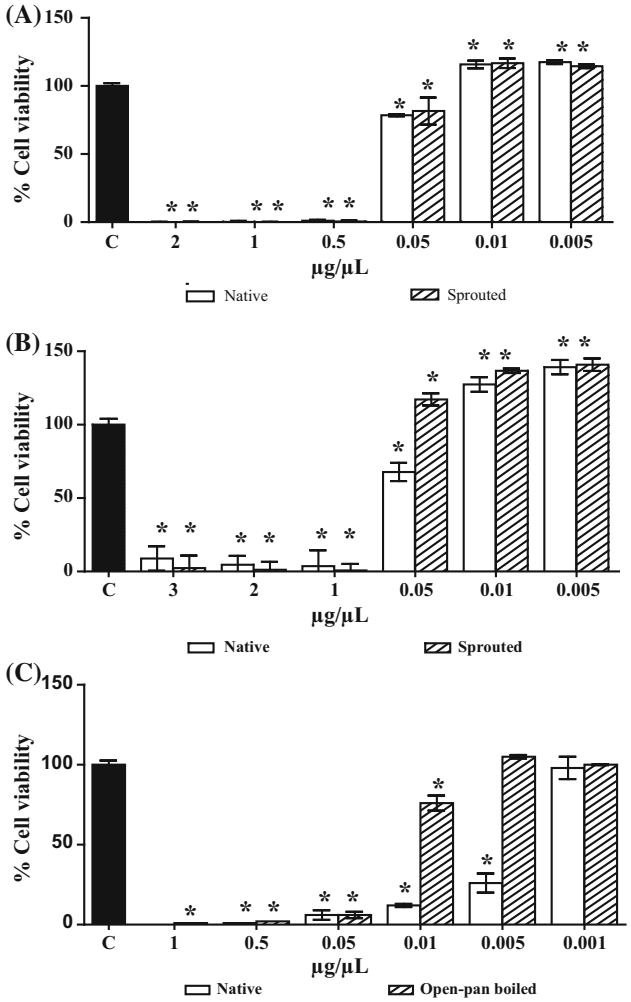

Figure 2. Viability of Caco-2 cells in the presence of phenolic compounds at various concentrations from (**A**) finger millet (0.005 to 2 µg/µL), (**B**) green gram (0.005 to 3 µg/µL), and (**C**) onion (0.001 to 1 µg/µL). Values are mean ± SD of quadruplicates. *Significantly different from control ($P < 0.05$).

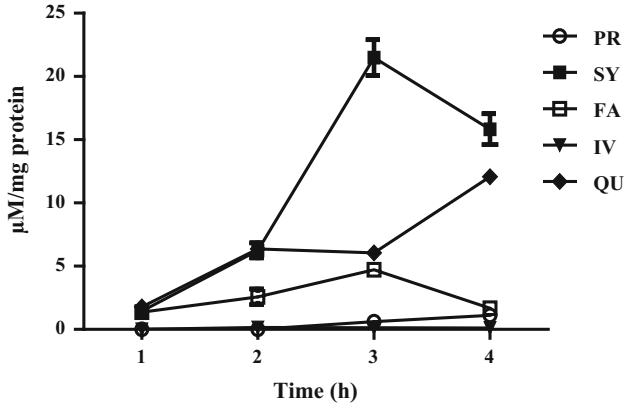

Figure 3. Uptake of pure phenolic compounds by Caco-2 cells at various time intervals (PR: protocatechuic acid; SY: syringic acid; FA: ferulic acid; IV: isovitexin; QU: quercetin). Concentration of PR and IV: 50 µM, SY, FA and QU: 100 µM. Values are mean ± SD of triplicates.

intervals is shown in figure 4A. Uptake of syringic acid, which was only 4.94 ng/mg protein at 1 h, increased by 50% in the next hour and reached a maximum of 19.4 ng/mg protein at 3 h and subsequently decreased to 13.0 ng/mg protein at the end of 4 h. The uptake of syringic acid was higher when sprouted finger millet was added to cells, as compared to native grain. Uptake of syringic acid from sprouted finger millet by the cells reached 18.8 ng/mg by 2 h itself.

Uptake of syringic acid by Caco-2 cells from digested native as well as sprouted green gram at different time intervals is shown in figure 4B. Cellular uptake of syringic acid from native green gram was 0.62 ng/mg protein at 1 h. Maximum uptake of syringic acid (1.48 ng/mg protein) from native grain by the cells was seen at 2 h, while it did not further increase any significantly at 3 and 4 h. Uptake of syringic acid from sprouted green gram by Caco-2 cells was higher than from native grain; the uptake of the same increased with time and was 2- and 3-fold at 3 and 4 h, respectively, when compared to 1 h.

Cellular uptake of quercetin from digested native as well as open-pan boiled onion is given in figure 4C. Quercetin was bioavailable from native onion after 2 h which increased by 5-fold at 3 and 4 h (0.15 ng/mg protein). Uptake of quercetin from open-pan boiled onion by the cells was only 0.04 to 0.069 ng/mg protein and was seen only up to 3 h. About 20% decrease in the uptake of quercetin from open-pan boiled onion was seen at 3 h.

3.3 *Transport of incubated phenolic compounds across intestinal Caco-2 cells*

Transport of standard phenolic compounds (protocatechuic acid, isovitexin, syringic acid, ferulic acid and quercetin) across Caco-2 monolayers is shown in figure 5. Transport of protocatechuic acid throughout the incubation period was below 20%. Maximum transport of protocatechuic acid across Caco-2 cells was seen at 1 h, while at 4 h it reduced to only 4.2%. Transport rate of syringic acid was up to 8.6% at 2 h but increased significantly at 3 h to 59%. Percent of ferulic acid that was transported across Caco-2 cell monolayer was below 19% throughout the experimental period. There was no significant difference in the percent ferulic acid transported. The amount of isovitexin transported from apical to the basolateral chamber by the Caco-2 cells was maximum at 2 h which decreased to 37% and 57% at 3 and 4 h respectively. Percent of quercetin transported was highest at 3 h (94.9%), however, decreased to 48.2% by 4 h.

Apparent permeability coefficient $P_{(app)}$ of phenolic compounds in Caco-2 cells is shown in figure 6. Highest permeability of 8.94×10^{-6} cm/s in Caco-2 cells was recorded by protocatechuic acid followed by ferulic acid (8.39×10^{-6} cm/s). Quercetin showed lower permeability value of 3.02 when compared to other phenolic compounds.

4. Discussion

Food grains and vegetables are a good source of bioactive polyphenols (Manach *et al*. 2004). The bio-efficacy of these phenolic compounds *in vivo* depends on the extent of their bioavailability, and hence an evaluation of the same is warranted. In continuation of our earlier reports on the bioaccessible phenolics content, we have measured here the uptake of several individual phenolic compounds by the Caco-2 cells, which show phenotypical similarities to the human small intestinal epithelium. In addition, transport of individual phenolic acids and flavonoids across Caco-2 monolayer was investigated. We also analysed the uptake of phenolic compounds from native as well as processed finger millet, green gram and onion. In a recent study, a similar protocol has been carried out to examine the bioaccessibility of carotenoids as well as polyphenols from plum and cabbage varieties (Kaulmann *et al*. 2016).

Protocatechuic acid and isovitexin above 50 µM concentration and syringic acid, ferulic acid, and quercetin above 100 µM concentration exhibited the cytotoxic effect on Caco-2 cells, which are nothing but human epithelial colorectal adenocarcinoma cells. Digesta obtained from finger millet and green gram showed the cytotoxic effect on Caco-2 cells up to 0.01 µg/µL concentration. This property of phenolic compounds can be utilized to study their probable anticancerous effect. Onion also exhibited a similar effect, but the extent of cytotoxicity was even greater when compared to other food samples studied, which may be due to the presence of quercetin, which at higher concentrations induces apoptosis and necrosis as reported (Jakubowicz-Gil *et al*. 2008).

Uptake of phenolic compounds by the Caco-2 cells varied at different time intervals. Uptake of isovitexin by the Caco-2 cells was very low when compared to other phenolic compounds. Also, poor gastrointestinal absorption of isovitexin in its actual form, than that of apigenin, *in vivo* is reported (Zhang *et al.* 2007). Probable intestinal degradation of flavonoids in the prevalent mild alkaline conditions has been documented (Xiang *et al.* 2017). An earlier report showed a better absorption of caffeic acid when compared to chlorogenic acid, as there existed monocarboxylic acid transport (MCT) along with the paracellular diffusion mechanism (Lafay and Gil-Izquierdo 2008). Highest uptake of 21.5% was recorded for syringic acid when compared to other phenolic compounds. Uptake of phenolic compounds was measurable

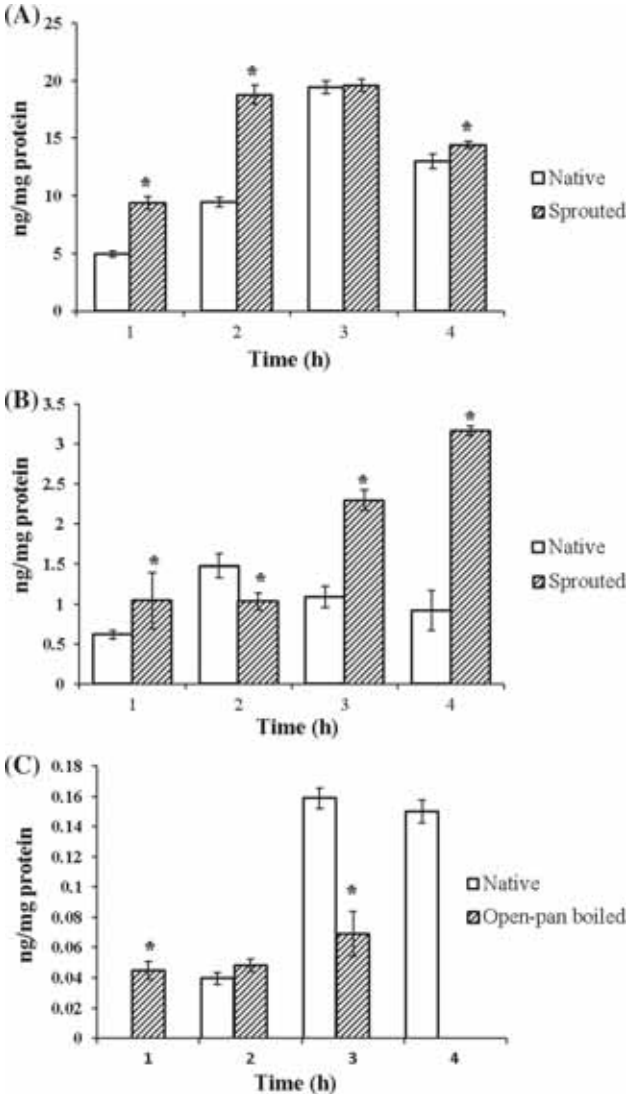

Figure 4. Uptake by Caco-2 cells of (**A**) syringic acid from finger millet, (**B**) syringic acid from green gram, and (**C**) quercetin from onion at various time intervals. Values are mean ± SD of triplicates.*Significantly different from native sample ($P < 0.05$).

at 2 h and the uptake of all phenolic compounds except ferulic acid and quercetin decreased by 4 h. The decrease in the uptake of phenolic compounds at 4 h may be due to the efflux of these compounds by the cells with this prolonged incubation.

Although many bioaccessible phenolic compounds have been reported from finger millet, green gram and onion (Hithamani and Srinivasan 2014a, b, 2016a), low concentrations of digesta of these samples, used in view of cytotoxicity, became the limiting factor for the detection of all the bioavailable phenolic compounds in the present study. *p*-Hydroxy benzoic acid and caffeic acid from native finger millet were found to get absorbed into the cells up to 4 h,

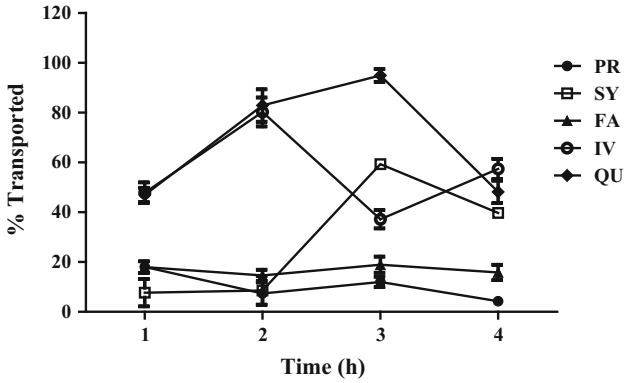

Figure 5. Transport of pure phenolic compounds across Caco-2 cell monolayers as a function of time (PR: Protocatechuic acid; SY: Syringic acid; FA: Ferulic acid; IV: Isovitexin, QU: Quercetin). Concentration of PR and IV was 50 μM and of SY, FA and QU was 100 μM. Values (mean ± SD of triplicates) are expressed as % of phenolic compounds in receiver compartment.

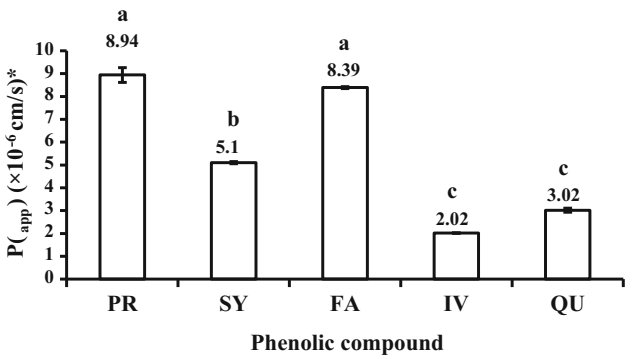

Figure 6. Apparent permeability coefficients $P_{(app)}$ of phenolic compounds in Caco-2 cells. *Transport of the phenolic compounds from apical to basolateral chamber (PR: protocatechuic acid; SY: syringic acid; FA: ferulic acid; IV: isovitexin, QU: quercetin). Values are mean ± SD of triplicates. Values not having similar superscripts in the same column are significantly different ($P \leq 0.05$).

while gallic acid was found only at 1 h (data not shown). As seen in our previous reports, sprouting enhanced the bioaccessibility of phenolic compounds from both finger millet (166 μg/g from 98 μg/g) and green gram (252 μg/g from 114 μg/g). Similarly, the uptake of syringic acid, which is a common phenolic acid of both the grains, was found to be enhanced on sprouting the grains. Open-pan boiling had a negative effect on the quercetin content, and hence its uptake reduced as compared to the native onion. It has been evident from a previous study (Price *et al*. 1997) that the content of quercetin reduces on cooking. It has also been reported that the food matrix has an impact on the bioaccessibility of the phenolic compounds; for instance, gallic acid was highly permeable from the food matrix rather than in isolation (Jiamboonsri *et al*. 2017).

Transport experiments on phenolic compounds evaluated the permeability of the same from apical chamber to the basolateral chamber. The permeability of phenolic acids across cell monolayer was more when compared to the flavonoids isovitexin and quercetin. As per apparent permeability coefficient $P_{(app)}$, ferulic acid and protocatechuic acid could be considered as more absorbable phenolic acids than syringic acid. These values correlated well with the fact that the permeability of molecules through a cell membrane can be well described as a linear function of the partition coefficient with slope dependent on the size of the molecule. Absorption also depends on the physicochemical nature of phenolic compound as evident by an earlier report (Rastogi and Jana 2016). It has been reported that transepithelial transport of ferulic acid was by mono-carboxylic acid transporter in Caco-2 cell monolayers (Konoshi and Shimizu 2003).

Percent transportation of flavonoids from apical to basolateral compartment was more when compared to the phenolic acids. Increase in the transport of quercetin from apical to basolateral compartment, up to 3 h and gradual reduction of the same shows the possible existence of a transport efflux mechanism for quercetin, which is supported by an earlier report (Borrás-Linares *et al*. 2015). Quercetin, the major flavonoid in onion (Caridi *et al*. 2007), exhibits neuro protective effect in rats (Pu *et al*. 2007). Flavonol quercetin is also known to possess cardio protective (Graf *et al*. 2005) and anti-inflammatory (Guardia *et al*. 2001) properties.

5. Conclusions

The study suggests that the absorption into intestinal cells occurs to a different extent for each phenolic acid; the uptake into the intestinal cells being very poor in a few cases. Available information is very limited to explain the differences among the uptake of phenolic acids and flavonoids. Obvious differences in the chemical structures of these phenolic compounds could cause differences in their absorption. On the whole, the present investigation suggests that the phenolic compounds studied are well absorbed in the Caco-2 cells and hence are available to exert their health beneficial physiological influences. Syringic acid was the major phenolic compound from both finger millet and green gram that was found bioavailable in the Caco-2 cells, and was revealed by our previous bioaccessibility studies as well (Hithamani and Srinivasan 2014a, b). Sprouting enhanced the uptake of syringic acid by Caco-2 cells from finger millet and green gram. Open-pan boiling of onions decreased the uptake of quercetin from onion by the Caco-2 cells. Further, *in vivo* studies are required to validate the bioavailability of phenolic compounds abundantly provided from food grains and vegetables.

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