Polyphenols are phytochemicals abundantly found in fruits, vegetables such as onion, tea and seed coat of food grains and are an important component of human diet because of their multiple health beneficial influences. Diverse physiological effects of phenolic compounds include: anti-platelet aggregation, blood cholesterol lowering, anti-inflammatory, cardioprotective and cancer preventive potential. Major classes of polyphenols are phenolic acids, flavonoids and tannins. To appreciate the potential of polyphenols in human health, we need to know the amount of polyphenols consumed in our daily diet and their bioavailability. Understanding the physiological influences of polyphenols requires knowledge of their intake, bioavailability and metabolism.

Traditional Indian foods being essentially plant derived provide rich amounts of polyphenols, due to the extensive use of fruits, vegetables and whole grains. The present investigation was carried out to evaluate the bioavailability of polyphenols from a few food grains (cereals and pulses) and onion. We particularly studied the effects of domestic processing methods (sprouting, roasting, pressure-cooking, open-pan boiling, and microwave-heating) on polyphenol bioavailability, using an appropriate in vitro model.

Content and bioaccessibility of polyphenols from cereal grains—wheat (Triticum aestivum), sorghum (Sorghum bicolor), finger millet (Eleusine coracana) and pearl millet (Pennisetum glaucum) as influenced by domestic processing were evaluated.

Total polyphenol content of wheat and sorghum was 1.20 and 1.12 mg/g respectively, which was increased by 49% and 20% respectively, on roasting. In contrast, a significant reduction of the same was observed in both the cereals after pressure-cooking, open-pan boiling and microwave heating. Total flavonoids that was 0.89 mg/g in native sorghum, reduced drastically after processing. Tannin content of both the cereals significantly increased on sprouting as well as roasting.

Bioaccessible total polyphenols from wheat was higher than that of sorghum. Domestic processing of these grains had minimal/ no effect on the bioaccessible total flavonoid content. Not all the phenolic compounds present in them were bioaccessible. Concentration of bioaccessible phenolic compounds increased especially on sprouting and roasting of these grains.

Total polyphenols of native finger millet was 10.2 mg/g which reduced by 50% after sprouting or pressure-cooking, while 12–19% reduction was seen after open-pan boiling. Total flavonoids of the grain reduced drastically on sprouting, pressure-cooking or open-pan boiling. Concentration of phenolic acids generally increased during sprouting and roasting of finger millet. Significant reduction of
total polyphenols was observed in pressure-cooked, open-pan boiled and microwave-heated pearl millet. Concentration of sinapic and salicylic acids were highest phenolic acids of pearl millet. Total polyphenols reduced during sprouting and pressure-cooking.

- Pressure cooking, open-pan boiling and microwave-heating reduced the bioaccessible polyphenols by 30–35%, while the same was increased by 67% by sprouting. There was a 20% increase in the bioaccessible polyphenols after sprouting of pearl millet. Thus, sprouting and roasting provided more bioaccessible phenolics from these cereals and millets studied.

- Food acidulants – lime juice and amchur, commonly used in Indian culinary were evaluated for their influence on the bioaccessibility of polyphenols from finger millet and pearl millet both in native and in processed conditions.

- Bioaccessible flavonoids increased by 25% in the presence of lime juice in roasted finger millet, while there was no significant change in total bioaccessible polyphenol content in pressure-cooked, open-pan boiled and roasted finger millet in presence of these food acidulants. Addition of amchur to pressure-cooked and microwave-heated pearl millet, increased the bioaccessible flavonoid content by 30% and 53%, while addition of lime juice increased the same by 46% in pressure-cooked pearl millet.

- Increased bioaccessibility of specific phenolic acids from finger millet and pearl millet was observed upon addition of these food acidulants. Thus, food acidulants–lime juice and amchur had significant influence on the bioaccessibility of health beneficial antioxidant phenolic compounds from the common millets.

- Content and bioaccessibility of polyphenols from legumes – Green gram (Vigna radiata) and chickpea (Cicer arietinum) as influenced by domestic processing were evaluated.

- Total polyphenol content reduced by 31% on sprouting but increased to 24% on roasting green gram. Pressure-cooking (53%), open-pan boiling (64%) and microwave heating (>2-fold increase) significantly increased total polyphenol content in chickpea, while drastic reduction was observed in the total flavonoid content.

- Bioaccessible total polyphenols from green gram was more than chickpea. Domestic processing of these grains had minimal/ no effect on the bioaccessible
total flavonoid content. Not all the phenolic compounds present in them were bioaccessible.

- Concentration of bioaccessible phenolic compounds increased especially on sprouting and roasting of green gram, while sprouting significantly reduced the same (631 to 351 µg/g) in chickpea. Microwave heating significantly enhanced the concentration of bioaccessible polyphenols from chickpea. Thus, sprouting and roasting provided more bioaccessible polyphenols from the legumes studied.

- The influence of food acidulants—lime juice and *amchur* on the bioaccessibility of polyphenols from legumes—green gram and chickpea both in their native as well as processed forms was evaluated.

- Addition of either lime juice or *amchur* did not significantly influence the total polyphenol content of native and roasted green gram. However, both lime juice and *amchur* increased the total flavonoid content in native green gram. Bioaccessibility of vitexin and isovitexin, which are the major flavonoids of green gram did not vary significantly in the presence of food acidulants.

- Bioaccessible flavonoid content increased 2-fold on addition of lime juice and >3-fold on addition of *amchur* from native chickpea. Gallic acid concentration in chickpea increased significantly after adding either lime juice or *amchur*.

- Presence of food acidulants—lime juice or *amchur* increased bioaccessible total polyphenols and flavonoids from both the legumes. This could strategic to derive health beneficial antioxidant phenolic compounds maximally from food legumes.

- Onion (*Allium cepa*) was analyzed for polyphenol and flavonoid contents and their bioaccessibility as influenced by domestic heat processing and presence of food acidulants. Total polyphenols of onion which were 2.17 mg/g were increased by 50% upon roasting, while total flavonoids (0.27 mg/g) did not change on heat processing.

- Bioaccessible polyphenols and flavonoids from onion were 0.96 and 0.02 mg/g, respectively. Open-pan boiling increased the bioaccessible polyphenols from onion. Addition of food acidulants to onion altered the composition and concentration of phenolics. Total bioaccessible polyphenols of onion decreased (by 15%) in presence of lime juice, while *amchur* increased the bioaccessible polyphenols by 21% in microwave-heated onions.
Bioaccessible polyphenols decreased in presence of lime juice both in native and pressure-cooked onion. About 37% increase in the same was noticed in roasted onion upon addition of amchur. Bioaccessibility of quercetin increased by 6-fold in the presence of amchur. Protocatechuic acid, syringic acid, rutin and myricetin were bioaccessible in presence of these food acidulants. Amchur enhanced the bioaccessible polyphenols from onion more than lime juice. Increased concentration of bioaccessible polyphenols was obtained on open-pan boiling of onion.

There was a qualitative as well as quantitative change in the phenolic composition on addition of the food acidulants. Since amchur enhances the concentration of bioaccessible polyphenols more than lime juice, its use in food preparations could be a strategy to maximize bioavailability of polyphenols, especially flavonoids from onion.

Uptake/absorption of polyphenols from plant foods was also studied in human intestinal Caco-2 cells. Absorption and permeation of pure phenolic compounds—protocatechuic acid, syringic acid, ferulic acid, isovitexin and quercetin was investigated in human Caco-2 monolayer model. Uptake of inherent polyphenols by Caco-2 cells from food grains—finger millet and green gram and onion was also studied.

Protocatechuic acid and isovitexin at >50 μM concentration and syringic acid, ferulic acid and quercetin at >100 μM concentration were found to be cytotoxic for these cells. Highest absorbed phenolic compound by the Caco-2 cells was syringic acid, while lowest was the flavonoid isovitexin. Significant uptake of both phenolic acids and flavonoids was observed after 2 h. Sprouting of grains enhanced the uptake of syringic acid by the Caco-2 cells from finger millet and green gram. Open-pan boiling drastically reduced the uptake of quercetin from onion.

Apparent permeability coefficient $P_{(app)}$ of phenolic compounds ranged from 2.02 ± 0.01 X 10^-6 cm/s to 8.94 ± 0.32 X 10^-6 cm/s. Permeability of phenolic acids across Caco-2 cell monolayer was in the order: protocatechuic acid >ferulic acid >syringic acid, and was more when compared to that of flavonoids which was in the order: quercetin >isovitexin. Phenolic compounds studied were able to cross the Caco-2 monolayer. Human intestinal Caco-2 cells served as a suitable model to study the uptake of phenolic compounds in isolation as well as from the food samples.

Thus, syringic acid was the major phenolic compound from both finger millet and green gram, found bioavailable in the Caco-2 cells as revealed in in vitro
experiments. Sprouting enhanced the uptake of syringic acid by Caco-2 cells from finger millet and green gram. Though, open-pan boiling did not alter the quercetin content *in vitro*, the same decreased its uptake from onion by the Caco-2 cells.

- Bioavailability of polyphenols from a representative cereal grain- finger millet, which is also a rich source of the same was investigated *in vivo* in rat model. The effect of co-administration of a known bioavailability enhancer ‘piperine’ on the absorption, tissue distribution and retention of orally administered finger millet polyphenols was also evaluated.

- Finger millet phenolic extract was orally administered (100 mg phenolics/kg) to rats. Another set of animals received finger millet phenolic extract concomitant with piperine (20 mg/kg). Blood, liver, intestine, kidney, and brain were analyzed for the absorbed polyphenols at varying time intervals (2, 4, 8, 16, 24, 48, 96 h) after the administration. Excretion of the absorbed phenolics through urine was also monitored.

- Concentration of polyphenols in plasma was maximal at 8 h after oral administration of finger millet polyphenols (13 µg/mL) with salicylic acid as the predominant polyphenol. Area under the curve (AUC) for phenolic concentration in plasma significantly increased (166 µg.mL⁻¹.h from 113) when piperine was concomitantly administered while AUC for individual polyphenols was also significantly increased.

- Finger millet phenolic compounds were distributed in various tissues as a function of time. Concentration of polyphenols in liver, kidney, small intestine, and brain increased significantly when co-administered with piperine at all the time intervals as reflected in the AUC values. Appearance of highest phenolic concentration in plasma, small intestine and kidney following the oral administration of finger millet phenolics was quicker when the same was co-administered with piperine.

- Finger millet derived phenolics were detected in brain at all intervals up to 96 h, and their concentration was higher when fed along with piperine. Urinary excretion of absorbed finger millet phenolics was significantly higher (302 µg) when co-administered along with piperine, presumably because of higher absorption.

- Thus, piperine co-administration significantly increased the absorption of finger millet polyphenols as well as their distribution and retention in different tissues following oral administration.
Conclusions:

It was confirmed from this investigation that whole grains and also onion are good sources of phenolic compounds. Though these food materials provided good amount of antioxidant phenolic compounds, not all the phenolic compounds were bioaccessible from them.

Domestic processing brought about qualitative as well as quantitative changes in the phenolic composition of whole grains and onion. Sprouting and roasting, especially enhanced the bioaccessible phenolic compounds from whole grains.

Addition of food acidulants to whole grains and onion made most of the phenolic compounds bioaccessible. Sprouting also increased the absorption of phenolic compound, especially syringic acid from grains, by the Caco-2 cells.

Higher amount of urinary excretion of syringic acid in rats, as compared to other phenolic compounds could suggest that, it is the more absorbable phenolic compound from finger millet.

By virtue of its active principle piperine, black pepper, when used in food preparations improves the bioavailability of health beneficial phenolic compounds.

oo O oo