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Bioavailability of Phytochemicals

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1. Introduction

Phytochemicals are increasingly accepted as health promoting, maintaining, and repairing agents in cells, tissues, or the whole human body. Phytochemicals are compounds obtained from plants that exert particular health effects; generally, they are not necessarily basic nutrients (minerals, vitamins, carbohydrates, proteins or lipids), medicines or toxins. The phytochemicals that are frequently associated with human health are phenolics, carotenoids, organic acids, and several miscellaneous bioactive compounds such as saponin and sterols. The contributions of phytochemicals in public health cover various issues world-widely and thus it is seen by researchers, industries, general society, and policy makers as a new tool to manage public health. Ironically, the roles of phytochemicals in health are poorly understood, which warrant the needs for validation as well as scientific database on safety issues and mechanisms of the functions. Even though various genetic-base studies propose mechanisms and health interventions of phytochemicals (Noe et al., 2004), many findings are inconclusive. Hence, the emerging health potentials of phytochemicals are inconclusive; and internationally it has been the reason for new policies/regulations in food trading. This is partly due to limited understanding on phytochemical bioavailability by which the health benefits depend on. Moreover, transport mechanisms for phytochemicals delivery into the target sites, phytochemical metabolisms by the human body, and biomarkers exerting the health benefits are also poorly understood. These complexities call for a new framework on how and to what extent dietary phytochemicals should be recommended in order to reach biologically-safe active dosages.

In the human body, bioavailability is defined as substances obtained from ingested materials that reach circulatory system for further delivery into designated tissues so that the beneficial compounds are biologically available for exerting healthy functions. The normal routes of dietary phytochemicals thus include ingestion, digestions, and transport across gastrointestinal epithelium prior to circulatory vessels. The epithelium in the gastrointestinal tract is a polarized enterocyte cell having two different sides facing luminal hollow (Apical side) and blood capillaries (Basolateral side) where each side is equipped with different transport facilities and barriers. The epithelial cells are critical for bioavailability of target compounds either as entrance gates or as metabolizing machines which release different compounds from the parent molecules. These make further complexing bioavailability routes because the metabolisms and transport processes are also

involved in the orchestrated physiological regulations maintaining homeostasis states of the human body. However, bioavailability of phytochemicals by which the health benefits depend on are not well understood; consequently, it is difficult to be measured.

The difficulties in studies of bioavailability are mainly due to the complexities involved in the biological system, i.e. (a) variation in food materials and the human subjects or surrogate models which are not always representative; (b) complex interactions amongst huge chemicals/food components during postharvest, storage, processing, digestion, and absorption that may alter health benefits; and (c) mechanism pathways. In this paper, fundamental aspects of phytochemical bioavailability are reviewed.

2. Digestibility of phytochemicals

It is known that major phytochemicals are located inside vacuoles of plant cells; and several phenolics form complexes with fibres in the cell wall. These natural existences make the phytochemicals poorly accessed by enzymes or hardly released out from the plant matrices during digestion. Most cell wall materials are indigestible by human enzymic systems. Moreover, it is also poorly permeable for important molecules such as phytochemicals. Therefore, digestibility of the phytochemicals is of great interest, in particular, to reveal how the phytochemicals can affect human health and fight or prevent diseases if the phytochemicals are strongly contained in the food matrices.

2.1 Digestion: principles of human gastrointestinal tract

The digestion compartments in human consist of mouth, gastric, small intestine, and colon (Figure 1). Each has slightly different digestion performances depending on age and gender as listed in Table 1. In the gastrointestinal tract, net nutriome¹ is released as a result of orchestrated secretions, enzymic activities, and physical-mechanical actions of peristaltic movements. The nutriomes will diffuse out from the food particles to chyme solutions. The levels of nutriome in this stage are called availability or accessibility of the components. However, bio-/chemical degradations of the molecules can take place. Hence, digestibility will also provide metabolites/derivatives. Nevertheless, availability and accessibility parameters can only account for intact molecules but not the metabolites.

Architecture and material of the plant tissues is generally unfavourable for activities of enzymic system in the human gastrointestinal tract. As a consequence, limited cell contents of the ingested food materials are released into chyme solution in the gastrointestinal tract. Natural pores and plasmodesmatas may not play predominant roles in diffusion of the nutriome. Nevertheless, according to Stolle-Smits et al. (2009), natural matrices of tomato, mango, apple, and kiwi undergo galactan solubilisation during ripening stage; thus the release of nutriome can be altered. However, processing and chemical compositions of the food matrices themselves may change physicochemical environments of the chyme for nutriome mass transfer. The most recent finding indicates that ingested foods are necessarily designed such that the diffusion of the nutriome favours nutriome absorption by epithelial cells; even for phenolics, it requires lipid-complex called phytosome (Kidd & Head, 2005) to penetrate gut lining and to enter the circulatory system.

¹Nutriome is a term referring to all beneficial food components

Digestion sites	Infant	Adults	Elderly	Female	Male
Mouth	Improper chewing, imperfect salivary enzymes	Chewing, complete salivary enzymes	Improper chewing, may be with incomplete salivary enzymes	Chewing	Chewing
Gastric	Acid & pepsin digestion	Acid & pepsin digestion	Acid & pepsin digestion	Acid & pepsin digestion	Acid & pepsin digestion
Small intestine	Immature system	Complete tissues and enzymes	Complete tissues and enzymes	Hormonal related digestive secretion, undisturbed by reproductive cycles	Hormonal related digestive secretion , disturbed by reproductive organ cycles
Large intestine	Predominant Bifido bacteria	Lossing Bifido bacteria	Lossing Bifido bacteria	Lossing Bifido bacteria	Lossing Bifido bacteria

Table 1. Summary of digestion characteristics of infant, adult and elderly, female and male

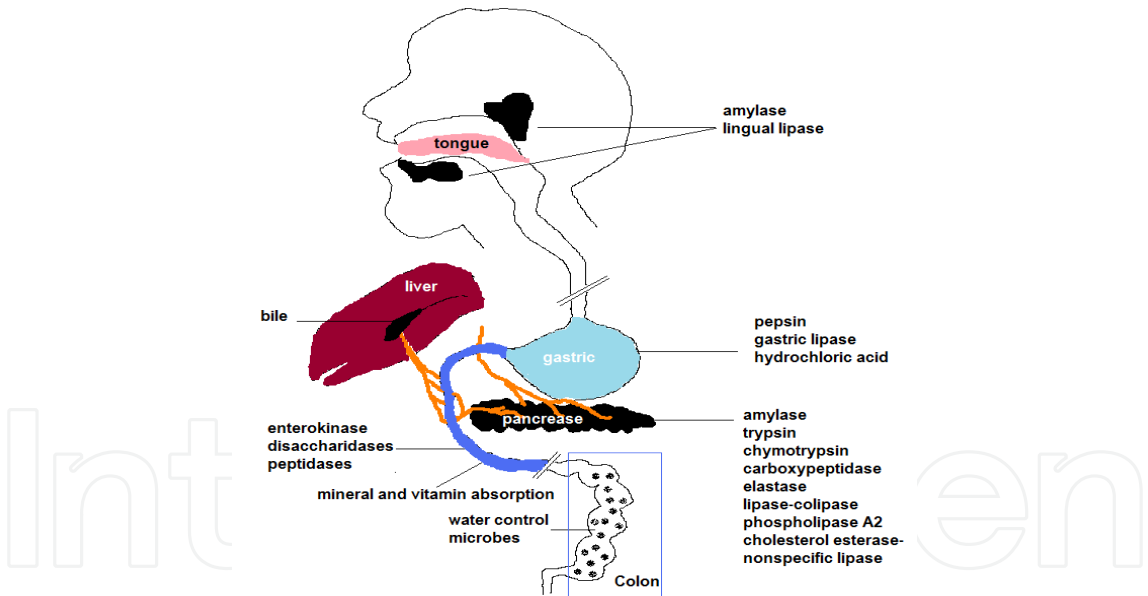


Fig. 1. Principles of human digestion system adapted and modified from Johnson (2001)

2.4 Effects of digestion on phytochemicals

Cell wall materials significantly modulate digestion of plant foods. Nunan et al. (1998) state that in grape berry during its development of the berry fruits the Na₂CO₃-soluble fraction increases before veraison but decreases as the berries softened. It implies that the Na₂CO₃ soluble fraction is the cell wall component which is responsible for firmness and strength. Epriliati (2008) observe that ripe mango, tomato, and papaya behave differently when Na₂CO₃ is added into *in vitro* digestion model mimicking small intestine where not all

aggregated boli from human *in vivo* chewing can be broken down. The diverse resistances of plant cell wall material amongst plant species during digestion may be partly due to Na_2CO_3 soluble fractions. It is more likely that mango and tomato have different levels of Na_2CO_3 soluble fractions which result in diverse *in vitro* digestion effects compared to papaya. Furthermore, processing will affect the way nutrime being released. Meanwhile, heating of the filtered fresh-juices (tomato, mango, and papaya) results in formation of clumpy substances (Epriliati, 2008). Similar clumps are also found as remnant of pectin gel used for taste masking agent of paracetamol and ambroxol (Miyazaki et al., 2005). These imply that consumption of fresh and processed various fruits, rich in pectin, can yield a wide range of phytochemical bioavailability depending on their cell wall material compositions. Furthermore, pasteurisation may render phytochemical release from the clumpy substances.

Phenolics. There are two main routes for digestion of dietary phenolics; i.e. digestion along the gastrointestinal tract and digestion inside the enterocytes. This can happen because hydrolase enzymes, i.e. lactase phlorizin hydrolase are available in intestinal lumen, brush border, and enterocyte (Williamson, 2004). Metabolisms that take place along the gastrointestinal tract are mainly aiming at deglycosilation of glycoside form of dietary phenolics. This deglycosilation is also carried out by microbiota in the colon.

Inside the enterocyte, dietary phenolic glucuronidation of the aglycone form are catalyzed by UGT². Meanwhile, the glycone forms are hydrolyzed and conjugated. The conjugated forms from both glycone and aglycone dietary phenolics are either effluxed into intestinal lumen or translocated into the portal blood vessel. The circulated conjugates of dietary phenolics in plasma can be absorbed by liver and hepatocytes will metabolize them further. For instance, the hepatocyte converts flavonoids into glucuronidated and sulphated forms, which are polar rendering to dissolve in water easier and then be excreted in urine or bile. The pivotal roles of liver indicate that these conjugations are apparently one of physiological needs in the body, for example for bile synthesis in mammals.

All compounds in wine, which are free from cell wall materials, show clearer responses during gastrointestinal digestion. Flavonol and proanthocyanidin interact with protein in the salivary secretion. However, catechin interacts stronger than epicatechin indicating that molecular characteristics play an important role in this interaction (de Freitas & Mateus 2001). Flavonols and proanthocyanidins remain intact but they may also be broken down when pH is sufficiently low in the stomach. Phenolics stability is strongly affected by pH as studied by Ginjom (2009). For example, syringic and *p*-coumaric acids are stable at pH 2-9 for 24 h. Generally, pH higher than 7.4 is unfavourable for phenolics and the effects of high pH are worsened by lengthy exposures. The number of -OH groups in benzene ring of simple phenolics can also be critical clues for phenolic stability. High pH results in unstable quinones which are oxidized further into diketones and other degradation products. In contrast, the stability of polyphenols such as quercetin, malvidin-3-glucoside, and resveratrol which have more than one benzene ring does not solely depend on their -OH groups. Quercetin is unstable during gastric and pancreatic digestions because quercetin is easily degraded at high pH, yet it is stable at pH 2 and pH 5.5 (Ginjom, 2009). In contrast, *trans*-resveratrol is stable at pH 1-7. Catechin isomers also show different stability at high

² UDP glucuronosyltransferase

pH as detected by Ginjom (2009). Similarly, (+)-catechin is stable in *in vitro* digestion up to pH 7.4 at 37 °C for 8 h different from that of (-)-catechin (Friedman & Jurgen, 2000 and Donovan et al., 2006). Overall, phenolics in wine do not undergo significant changes during gastric digestion.

In red wine, anthocyanidin is important component of phenolics. Anthocyanin availability is reduced by 32% after pancreatic digestion compared to that of gastric digestion and undigested sample (Ginjom, 2009). Pancreatic environment, however, decreases monomeric anthocyanin more severely (58.75%) than polymeric anthocyanins (17.72%). Pancreatic condition in the intestine modifies molecular structures of peonidin-3-glucoside, malvidin-3-glucoside, and malvidin glucoside pyruvate as indicated by the changes in their retention time during HPLC/UPLC analyses, although it is unclear why this can happen. However, during *in vitro* digestion, Ginjom (2009) speculates that the losses of monomer are related to their polymerization during the pancreatic digestion. Although non-anthocyanins are insignificantly affected by gastric digestion, pancreatic digestion severely reduces them by ca 88% (equivalent to 22% of total phenolics in red wine). On the other hand, flavan-catechin is speculated to polymerize with anthocyanins or tannin forming precipitates during pancreatic digestion; consequently, they either being eliminated during sample preparation or disposed in aqueous fractions.

Phenolics in tomato products are released into digest solutions more during *in vitro* gastric digestion than during pancreatic digestion and the highest release is from tomato juice (Epriliati, 2008). The main phenolics in tomato are caffeic, catechin, rutin, chlorogenic acid, and coumaric acids. More phenolics are obtained from tomato juice than those from dried and fresh tomato indicating the natural barrier of cell wall has been eliminated. Noticeable changes of phenolic compounds due to processing and digestion are found but the new compounds are not able to be identified. Rutin and catechin are consistently found in fresh, juiced, and dried products. Meanwhile, no *p*-coumaric is found in fresh product whereas *p*-coumaric gradually appears in juiced and in dried products. In contrast, chlorogenic acid is present in fresh products but it gradually disappears in juiced and dried products. This could be caused the different extractability due to different matrices of the products or by chemical changes due to processing and digestion environments (Epriliati, 2008). Gastric digestion does not affect phenolic compounds. However, the phenolic levels are significantly reduced in consecutive gastric-intestinal digestion. Apparently, tomato pectin neither gels nor traps phenolic compounds at lower pH. Altering pH from gastric to intestine may obstruct the molecular phenolic stability.

Similarly, there are different phenolics released from mango during *in vitro* digestion. The phenomena consistently support the possibilities of impermeable pectin where more phenolics are released in a consecutive gastric-intestinal digestion when aggregated boli can be broken down with the addition of $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ (Epriliati, 2008). Recently, phenolics in gastrointestinal tract markedly behave in a similar way to that of carotenoids incorporated in chylomicrons, thus, all emulsified phytochemical compounds are called phytosome³ (Kidd & Head, 2005). Therefore, the presence of pancreatic juices and bile extract improve phenolics release during consecutive gastric-intestinal digestions of mango.

³ Phytosome is a term for vehicles in which phytochemical compounds are bound

Carotenoids. About 50% of extractable carotenoids dominated by lycopene and β -carotene in tomato, mango, and papaya products are released to digest solution in a non-lipidic digestion model (Epriliati, 2008). The release of carotenoids increases significantly in intestinal digestion where bile extract and pancreatic secretions exist. Consecutive gastric-intestinal digestions do not help with higher release of carotenoids. This is more likely due to insufficient emulsifier-water ratios to provide emulsification of carotenoids which are fat soluble. It is concluded that mango, tomato, and papaya carotenoids are released better in intestinal digestion where the model is without addition of oil (Epriliati, 2008).

Organic acids. Pectin content in tomato hinders organic acid release thus the total organic acids in *in vitro* gastric digest solution is lower than that of consecutive gastric-pancreatic digestion. This is evidenced by the changes in pH from highly acidic gastric pH to higher small intestinal pH (~6), that causes disaggregation of boli during *in vitro* digestion. For all types of mango samples, organic acid including ascorbic acid (Vitamin C) is released better during gastric digestion. Apparently, the pectinous materials in mango do not trap organic acids (Epriliati, 2008).

3. Absorption of phytochemicals

Currently, there is no well-established molecular form of absorbed substances in the gastrointestinal tract, i.e. whether they are absorbed intact or as metabolites. On the other hand, it is well known that lifestyle, behavior, diets, and basal metabolism of the subjects are more important affecting factors than age, gender, body weight, and plasma volume (Manners et al., 2003) in bioavailability determination. Therefore, standardized experimental conditions controlling such critical factors of absorption *in vivo* and *in vitro* is a must despite individual human variability.

3.1 Absorptive tissue structures

The main absorptive tissue is the small intestine. In human 81% of the total intestinal lengths is by small intestine and 19% is large intestine. The stretched length of jejunum is around 30.78% of the intestinal lengths. The transit time along human small and large intestine is 3-4 h and 2-4 d, respectively (Vermeulen, 2009). Principles of the intestinal absorptive structures are depicted in Figure 2. A single enterocyte has microvilli and each microvillus has glycocalyx. Such structure considerably increases contact surface areas with luminal contents. Each microvillus also contains a complex structure providing various facilities for uptake/influx and efflux molecules, signalling ports, cytoplasm, and lipid matrix. The glycocalyx and microvilli are the areas where the human body depends on for collecting nutriomes but rejecting hazardous compounds including microbes.

Each enterocyte attaches onto adjacent cells through tight, adherence and gap junctions. Cellular transport from intestinal lumen to portal blood vessel occurs in two ways: paracellular and transcellular. The paracellular entrances for hazards are tightly controlled by those three types of junctions. Molecular weight cut off limits the hazardous substances crossing through both enterocyte lining cells and tight junction. The enterocytes collect compounds that reach apical side. The compounds then traverse into basolateral side where they end up in capillary vessel for circulation into the whole human body.

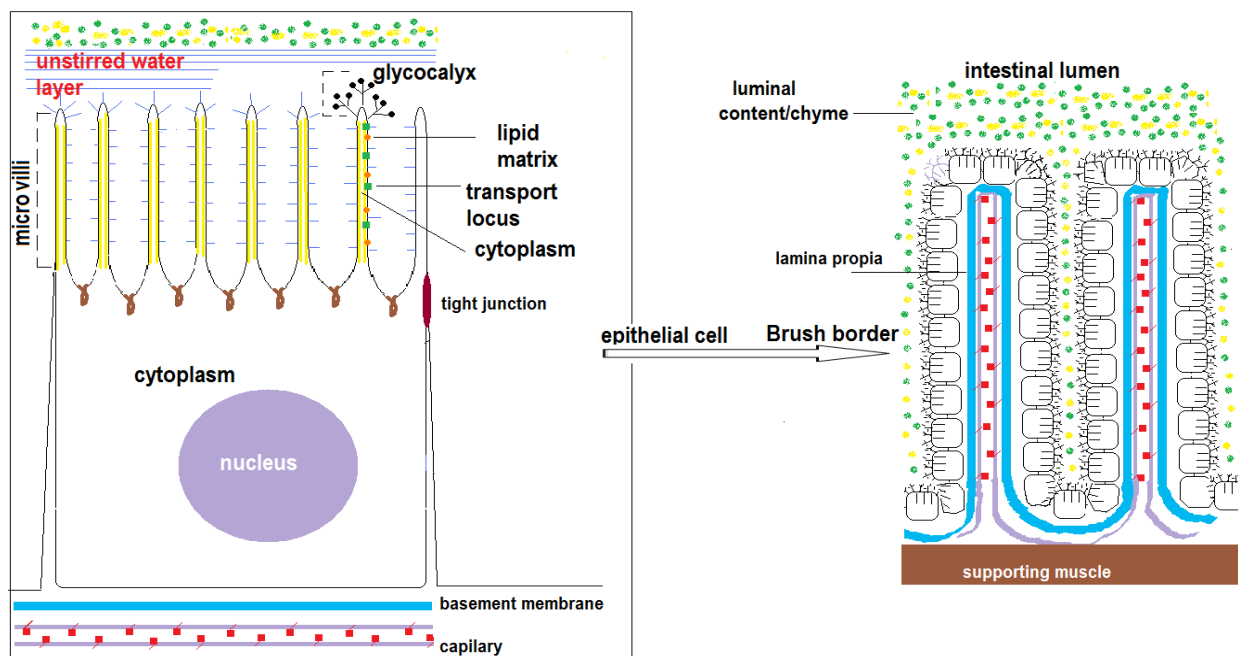


Fig. 2. Absorption tissue: epithelial cell (left) and intestinal brush border (right)

Other barrier in intestine is mucus. Most absorptive tissues comprise of epithelial cells which protect the human body from hazardous components in ingested foods. The epithelial cells are critical gate for human body. Due to its critical roles, the epithelial cells along gastrointestinal tract are covered with mucus secreted by goblet cells making an unstirred water layers so that the coarse particles are not abrasive towards the epithelial cells.

3.2 Transport mechanisms

Epithelial cell membrane is an important part of transport facility. It controls and selectively takes up molecules required for living or treats hazardous molecules. The fate of its work is not well understood despite studies finding many facilities and signaling processes available for regulating transport molecules. The transport modes include passive and active mechanisms. Passive transport is transcellular or paracellular transports and cytotocytosis. Active transports are characterized by the use of protein transporters: channels/pump, binding protein transport, and formation of vehicles that is mainly emulsion system incorporating oil soluble compounds, such as chylomicrons. The transporter is able to promote transmembrane movement without hydrolyzing ATP (Johnson, 2001). They are categorized as uniporter (single compound moving down along the electrochemical gradients) and symporter (two molecules at the same time moving in one direction) or antiporter (two molecules at the same time moving in opposite directions).

Several transporters act as cellular efflux port for flavonoids: *P*-glycoproteins, multidrug resistance associated proteins, and breast cancer resistance protein (Johnson, 2001). They generally have loose substrate specificity and also involve in regulating non-nutritional compounds. Several findings point out glucose transporters which allow quercetin glucoside to be absorbed intact besides its aglycone forms. They are SGLT1⁴ (SLC5A1⁵),

⁴ Sodium dependent glucose transporter

GLUT2⁶ (SLC2A2), MCT⁷, OAT⁸, and OATP⁹. However, results from the *in vitro* cell culture-based experiments are contradictory. Recently, bilitranslocase transport was introduced (Passamonti et al., 2009), that suggests the existence of a uniporter for flavonoids which is assumed to be an analogue of phthalien due to their similar molecular structures. The bilitranslocase is distributed in goblet and parietal cells in gastric, in apical jejunum of rat intestine, and in basolateral site of proximal tubular cell in kidney. However, further research is required for better understanding.

Briefly, bilitranslocase description indicates that target molecules interact with bilitranslocase through hydrogen bonds (hydrophilic properties of the active site); thus, nonionic inhibitors would not interact with it electrostatically. However, a negative charge is found to play an important role for electrogenic movement along the translocation pathway. These are observed through structural analysis (Passamonti et al., 2009). Similarly, the competitiveness of the target compounds can be explained by characteristics of C₄ in C-ring flavon building block where the target molecules are inactively competitive if the sugar moiety is in non-planar position; otherwise, the molecules will be actively competitive. Taking an example of quercetin-3-glucoside, the C₄ carbonyl forces 3-glucosyl moiety is perpendicular to the plane of flavonol aglycone resulting in a non-planar molecule. In contrast, its best competitor cyanidin-3-glucoside has a co-planar sugar moiety to the aglycone. Similarly, comparison of myricetin and delphinidin behave noncompetitively and competitively, respectively. Consequently, noncompetitor and competitor can be in one target molecule if its molecular structure has quinoidal, anionic tautomer, and neutral phenolics. They simultaneously can bind both the noncompetitive and competitive sites of bilitranslocase. Inconclusive role of bilitranslocation is compounded further by noninhibitor responses of other flavonoids, for instance flavonol (+)-catechin and isoflavones—genistin, genistein, daidzin, daidzein, and puerarin (Passamonti et al., 2009).

Bilitranslocase sheds a light for phenolics bioavailability and transport studies. The most striking relevance is that phenolics bioavailability is not delivered to blood circulation; instead, it is delivered through lymphatic system. This corrects understanding of hydrophobic nature of phytochemicals. The presence of bilitranslocase also clarifies disappearance of flavonoids in apical side but no basolateral level obtained in Ginjom (2009) and Epriliati (2008), despite GLUT2, MRP¹⁰, organic cation, and amino acid/peptide transporters are available in basolateral domain. This specificity is promising for explaining the diverse bioavailability studies of phytochemicals.

3.3 Phytochemical absorption

There are two groups of nutrime: water soluble and less polar-solvent soluble. The water soluble components diffuse out from the food particles into chyme, traverse across the epithelial lining cells along the brush borders, and enter the portal blood circulation. On the other hand, the lipid soluble nutrime will be emulsified by bile salts and lipidic

⁵ Solute carrier

⁶ Glucose transporter

⁷ Monocarboxylate transporter

⁸ Organic anion transporter

⁹ Organic anion transporting polypeptide

¹⁰ Multidrug resistance-associated protein

components of diets immediately after diffusing out from the food particles. The emulsion acts as vehicles moving along the intestinal lumen. Contacting with the epithelial brush border and unstirred water layer on the top of the epithelial lining cells, rearrangement of vehicle emulsion take place which eventually releases the lipid soluble compounds into the cells. These compounds then traverse across the epithelium cells and end up in the lymph circulation. Nevertheless, many studies show losses material balances during transport across the epithelial lining gut. Moreover, the proportion of traversing compounds which are both water soluble and lipid soluble nutriomes that survive intact entering the circulatory system is not well understood. Similarly, proportion of metabolized nutriome used up by the epithelial cells as energy source is unclear.

Phenolics. Many studies support evidences that aglycone polyphenols are not only absorbed in the small intestine but also in the large intestine after microbial digestions. The steps may involve hydrolysis of sugar moiety by intestinal enzymes.

In the human small intestine and stomach, 95% of caffeic acid is absorbed while 62% of its ester form (called chlorogenic acid) is reduced. All are absorbed intact, except chlorogenic acid which mostly enters the human body from colon. Proanthocyanidins are pH sensitive thus it is likely to be broken down in stomach so that they may be readily absorbable. Meanwhile, catechin and epicatechin is poorly absorbed in the small intestine ($\leq 20\%$) in a dose dependent manner. However, enterocytes can act differently; for instance, in intestinal jejunum it metabolizes flavanols into glucuronidated conjugates whereas in ileum it translocates flavanols intact. In the large bowel, most microflora metabolize flavonols and proanthocyanidins; for example, catechin metabolites include (-)-5[3'4'5'-trihydroxyphenyl]- γ -valerolactone; (-)-5[3'4'-dihydroxy phenyl]- γ -valerolactone; 3-hydroxyphenylpropionic acid; 3-hydroxybenzoic acid; or 3-hydroxyhippuric acid (Ginjom, 2009).

With a new bilitranslocase transport mechanism it is likely that the determinations of bioavailability of phytochemicals are necessarily being revised. pH and temperature are necessarily taken into account in order to avoid underestimation/overestimation regarding its stability. Several issues include absorption of quercetin and anthocyanin, glycone and aglycone forms, and conjugation/glucuronidation of phytochemicals as well as the presence of alcohol. Quercetin absorption varies from one food source to another. Its absorption from wine is enhanced by alcohol presence. Resident time of quercetin expressed as half-life clearance is 11-28 h (Manach et al., 2005). A very low level of intact anthocyanins is found in plasma after administration of anthocyanins. Resveratrol is absorbed well in the small intestine and being glucuronidated. Consumption of red wine would provide a good level of resveratrol bioavailability can be questioned whether this is because of alcohol presence.

Flavonoid is one of the group molecules with molecular weights >500 Da and has bioavailability level of $<1\%$. Such molecules are unlikely to be transported through passive diffusion pathways. Further study found that influx membrane transporters cannot recognize flavonoid (signalling) whereas the efflux transporters do. Consequently, potential of flavonoids to be expelled is higher than that of influxed into the cells (Johnson, 2001).

In determination of phytochemical bioavailability, researchers should not limit their detection for ingested molecular forms only based on reported presence in the diets. It has been proven that at plasma levels many phytochemicals have been conserved by digestion and by hepatic activity. Fitting the mass balance of ingested phytochemical is challenging.

For instance, total metabolites in plasma levels are found reaching 4 mmol/L when intake is 50 mg aglycone equivalent whereas urinary excretion levels are 0.3-43% of the ingested doses, depending on polyphenol types. Flavonol such as quercetin in broccoli is rarely found as free quercetin. Human who consume 21-100 mg/d of quercetin show exclusive form of methyl, sulphate, or glucuronic acid conjugates totally amounted to maximum 1-5 μ mol/L aglycone equivalent (Moreno et al., 2006). However, several phytochemicals are found intact, especially those which are absorbed easily. The ranks of phytochemical absorption is gallic acid and isoflavones > catechins and flavanones, quercetin glucoside > proanthocyanidins, galloylated tea catechins, and anthocyanins (Moreno et al., 2006).

Carotenoids. Carotenoids of mango, tomato, and papaya in caco-2 absorption model are not detected (Epriliati, 2008) in spite of *in vivo* data indicates that carotenoid plasma level increase after consumption of carotenoid-rich foods. Processing altered matrices of ingested food system and more likely degraded carotenoids which caused variation in bioavailability of carotenoids. A comparative study of organic and inorganic carrot found that apparently organic farming practices do not affect bioavailability of carotenoids in carrot consumption. Ingestion of total carotenoids of 24.3 ± 1.4 mg organic carrot and 23.2 ± 2.5 mg inorganic carrot results in 700 nmol/L β -carotene and 350 nmol/L α -carotene, and 150 nmol/L lutein after 2 weeks interventions (Stracke et al., 2009).

Organic acids. Organic acid provides organic anion important for metal binding and counteracting acidosis as well as preventing chronic diseases (Sabbah et al., 2011). Particular organic acids are apparently absorbed into plasma. Most organic acids in tomato, mango, and papaya products are absorbed in *in vitro* caco-2 model but they are not found in the basolateral sides (Epriliati, 2008). On the other hand, citric acid and oxalic from banana and sweet potato are consistently found to be absorbed and translocated into basolateral sides in *in vitro* caco-2 model (Sabbah et al., 2011). The absorbed organic acids are much lower compared to the original levels in food materials, thus, the retained organic acids in particles may be useful for controlling pH in colonic fermentation because selection of microbes in the large bowel is important.

Miscellaneous. Phytosterol could be absorbed at very low level using the same transport facilities for cholesterol due to structural similarities. It needs emulsion vehicle to diffuse in the aqueous lumen system, crossing the lipid membrane, and, finally, entering circulatory system. This requires evaluation because absorption is closely connected to which mechanisms are involved in health function, which is still debatable (Kang et al., 2010).

Triterpenoids citrus limonin glucoside is one of metabolites in citrus plant. Generally, it is water soluble; yet few aglycone forms of limonoids are insoluble. According to Manners et al. (2003) limonoid metabolites are found in human after ingestion of citrus juice containing limonin glucoside which may undergo epimerization from limonin glucoside to epilimonin (m/z 471.2). This may be from reaction pathways of hydrolyzation of glucoside moiety followed by lactonization. Although low level of limonin is ingested, it is eventually available in plasma after 6 h (Manners et al., 2003). During the first 3 h the higher ingestion level of limonin results in more significant changes in plasma epilimonin levels, regardless of age and gender. However, after 6 h, all volunteers show increased levels of plasma epilimonins at any ingestion levels of 0.25 g/200 mL–2 g/200 mL that is equivalent to 7 glasses of natural juices. The authors conclude that ingestion of limonin glucoside produces

epimer limonin at C₁₇. It is clear that the human body does not necessarily control levels of plasma limonin and its absorption in the gut whereas limonin glucoside enters blood plasma through GLUT pathways, but it is necessarily hydrolyzed and lactonized. If it is absorbed through GLUT pathways without being metabolized, it should enter blood plasma at the same rate with sugars. The problem is that variation of individuals cannot be ignored since by the time it shows accumulation or decrease of detected limonin levels. The consequence of this accumulation is also not understood. Overall, limonin aglycone form is apparently safer than that of limonin glucosides; therefore, the high level of limonin glucoside form is controlled. Based on transit time of chyme in the gut, 6 h will be long enough to bring the chyme completely passing the small intestine. Therefore, lower level of ingestion results in limonin absorption after microbial glucoside hydrolysis in bowel. These speculations remain to be elucidated.

Interactions involve in various phytochemicals and nutrient transports. Since phytochemical are generally reactive molecules they can interact with various compounds in the chyme and this will affect phytochemical bioavailability and vice versa. Phytochemicals that interact with vitamin E include lignans, curcumin, anthocyanins, phenolic acid and catechin, as well as cereal alkylresorcinol (Frank, 2004). Interaction of vitamin E and plant lignans significantly increases vitamin E bioavailability as much as 900% in plasma level; 1,350% in liver; and 1,556% in lung using rat model. On the other hand, using human and rat model tocopherol- ω -hydrolase activity is effectively inhibited by sesamin¹¹. Sesamin also reduces degradation of γ -tocopherol and urinary secretion so that it increases γ -tocopherol level in plasma. However, not all lignans show similar effects. For instance, sesamin or flaxseed lignan secoisolariciresinol diglucoside, either its monomer or oligomers decrease tocopherol by 50%. Experiment using rat model indicates that flaxseed lignan decreases α and γ tocopherol availability in a dose dependent manner. However, it presence increases lipid peroxidation. The majority of flaxseed lignan is converted into mammalian lignan allowing them to be absorbed (Frank, 2004).

In contrast, the effect of curcumin studied using rat model on α tocopherol bioavailability is less apparent when compared to flaxseed or sesame lignans where it is only detected in lung. In fact, curcumin is absorbed, metabolized, and secreted as glucuronidated metabolites. Similarly, the effect of anthocyanins on tocopherol bioavailability is neglected. Using the same rat model, it is found that caffeic acid increases γ -tocopherol in the liver and it is also converted into its metabolites 5-caffeoylquinic acid which in turn increases the levels of α -tocopherol in lung. However, when ingested as 5-caffeoylquinic acid, it is metabolized into caffeic acid and quinic acid; and caffeic acid is absorbed and found in plasma both in human and rat models. In contrast, ferulic acid is found to form complexes with albumin in blood plasma and LDL; hence, it does not affect tocopherol bioavailability. Interestingly, (+)-catechin and (-)-catechin isomers similarly improve α -tocopherol bioavailability in plasma and liver (Frank, 2004). There is a slight difference regarding their effects on γ -tocopherol where 2R,3R-isomer(-)-epicatechin enhances γ -tocopherol bioavailability whereas 2R,3R-(+)-catechin has no effect on it. The differences between γ - and α - tocopherol is estimated due to (i) antioxidant activity of catechin isomers on a tocopherol and (ii) different effects of the isomers on cytochrome P450 enzymes such as

¹¹ Lignan exists in sesamin

CYP1A1, CYP1A2, CYP2B1, AND CYP3A4 as well as CYP4F2. Alkylresorcinols in outer layer of wheat and rye is also absorbed and metabolized. Its presence improves γ -tocopherol in liver and lung but not α -tocopherol observed in rat. The various effects on tocopherol isomers are unclear although molecular differences of alkylresorcinol and tocopherol is known.

Addition of citric acid affects iron uptake. In reverse, citrate reduction improves iron bioavailability (Glahn et al., 1998). Iron bioavailability is also influenced by purple and brown pigments in rice; apparently, the pigment behaves similarly to tannin, phenolic, anthocyanin, or phytic acid (Glahn et al., 2002).

Interactions amongst carotenoids (Kostic et al., 1995; van den Berg, 1999) show that β -carotene inhibits lutein uptake. These interactions perhaps also occurred at the micelle formation and transport levels, or their combination (van het Hof et al., 2000). Similarly, β -carotene shows competitive inhibition to lycopene transport (Johnson, 1998). Meanwhile, carotenoids can interact with proteins and pectin decreasing absorption the carotenoids (Williams, 1998). Moreover, the catechol structure in the B-ring of flavonols and 2,6-di-*tert*-butyl-4-methylphenol inhibits the dioxygenase enzyme and conversion of β -carotene (Nagao et al., 2000; Nagao, 2004). On the other hand, metabolites of bio-oxidation may act as pro-oxidants in the body (Nagao, 2004). Konishi found that tea phenolics inhibit other dietary phenolics (Konishi et al., 2003).

Among several fruits and vegetables, papaya and tomato consumption are found to be beneficial in hypolipidemic diet components, with similar mechanisms observed during *in vivo* experiments using rats (Kumar et al., 1997). Here, soluble and insoluble fibers can bind bile acids, thus influencing micelle formation and absorption of lipophilic substances by the brush border. Lignin and guar gum are apparently better bile binders than cellulose, which is relatively inert. Interaction also occurs between fiber and intestinal mucin, which probably alters absorption and nutrient diffusion from bulk lumen content (Vahouny & Cassidy, 1985). Moreover, fiber bound health promoters include lycopene in tomato peel (Awad et al., 2002) and antioxidants in mango peel (Larrauri et al., 1996), where the antioxidants found in mango peel, pulp and seed include gallotannins (Berardini et al., 2004). Consumption of fiber-rich food products can reduce minerals and vitamin (Schneeman & Gallaher, 1985). Generally, those authors agree that pectin and cellulose play important roles, especially in reducing the activity of digestive enzymes, or hormones such as insulin (Schneeman & Gallaher, 1985; Vahouny & Cassidy, 1985).

4. Kinetics simulation of phytochemical bioavailability

Kinetics is a study observing changes of the phytochemicals after ingestion including elimination period. To understand kinetics of phytochemicals after ingestion, kinetics simulation is frequently carried out. The limitations of simulations should be acknowledged in interpreting the results. Moreover, bioavailability closely relates to absorption and metabolism, yet there are limited understanding of bioavailability markers. Furthermore, the markers need validating, i.e. the molecular forms selected as bioavailability markers are necessarily those which actually cause health effects.

Affecting factors of phenolic bioavailability include matrix of food sources, processing condition during food preparations, chemical compositions, and molecular physicochemical

properties of the target molecules. Molecular forms of phenolics such as glycone or aglycone definitely make diverse variations on bioavailability levels. In addition to these factors, individual gastrointestinal tract of the human also affects bioavailability. Gastrointestinal pH, level of secretions, microbiota, and age have been established as crucial factors affecting digestion and absorption of phytochemicals. Equally, the role of interactions amongst food components and their interactions with gastrointestinal secretions contribute significant effects in determining bioavailability of phytochemicals.

Tannin-protein interactions occur starting from mouth and food systems. The interaction depends on size, conformation, and charges of proteins; molecular size, flexibility, and water solubility of phenolics; and environmental conditions such as pH. Proteins with higher molecular weights or loose conformational structures or rich in proline/hydrophobic amino acids, increase its potential to be precipitated by tannin. On the other hand, flavonols (three orthohydroxyl groups on the B-ring) has higher affinity to protein compared to those with two orthohydroxyl groups. Similarly, the affinity increases with increasing galloylation degrees. The order of flavonols affinity is (-)-epigallocatechin gallate > (-)-gallocatechin gallate > (-)-epicatechin gallate > (-)-epigallocatechin or (-)-epicatechin or (+)-catechin (Ginjom, 2009). Interestingly, tannin also plays pivotal roles in its capability to act as health protective antioxidant.

4.1 *In vitro* model of digestion and transport

Effects of *in vitro* digestion on wine phytochemicals are significant during pancreatic digestion step, especially for nonpolar compounds. Therefore, water solubility level is crucial in generating an appropriate *in vitro* digestion model. In contrast, acid does not significantly affect the phytochemical components in wine.

In vitro model for absorption using a monolayer cell culture can help bioavailability determinations with human surrogates; however, the results should be carefully considered. More importantly, the results cannot be liberately generalized for human system biology. Yun et al. (2004) propose a constant to equalize *in vitro* measurement using caco-2 monolayer with human *in vivo* measurement for iron. Furthermore, there are critical factors in utilizing such *in vitro* model for a bioavailability study that should be carefully considered. For instance, the original composition of digest containing bile salts decreases TEER (transepithelial electrical resistance) indicating serious detrimental effect on the cell monolayer integrity. In addition, alcohol content in wine also affects the monolayer integrity so that alcohol removal is required although alcohol enhances phenolic absorption. This is unrealistic wine samples. Furthermore, the delicate properties of the monolayers may result from lacking of mucus/unstirred water layer protecting the epithelium. The development of an appropriate and standardized *in vitro* model needed to be persued continuously.

4.2 Kinetics of phytochemical bioavailability

Kinetics study of phytochemicals is scarce. Several experiments are reviewed below to understand phytochemical kinetics after ingestion.

Quercetin. Quercetin is more likely to be absorbed quickly in the human gut after ingestion, e.g. quercetin-3-glucoside from blackcurrant juice is 4 h or pure quercetin glucoside capsule is ca 30 min. Quercetin-3-rutinoside takes longer time to reach peak plasma levels compared

to the two previously mentioned, i.e. after 5-10 h. Short- and long- term studies show kinetics absorption of quercetin is quick and easy; and there are no interactions with other food components. Moreover, bioavailable quercetin can be obtained from normal diet regardless of whether it contains the berries or not. Therefore, it is proposed that fasting quercetin bioavailability is used as a biomarker of high fruit and vegetable intakes for all plant based foods (Erlund et al., 2006).

Soyasaponin. Soyasaponin has a very low bioavailability when investigated using *in vivo* experiments involving animals and human (Kang et al., 2010). However, it is also found that possible metabolites of soyasaponin are detected in *in vitro* and *in vivo* studies, although it is found several days after ingestion (Kang et al., 2010). The metabolites include soyasapogenol B, which is secreted into faeces in human *in vivo* experiments. However, the metabolism is more likely due to microbiota in the colon which is supported by *in vitro* data using fresh faecal microbiota. *In vitro* data show sequential metabolism of sapogenin by the microbiota as follows: soyasaponin I after 48 h incubation at 37 °C, and it is converted into soyasaponin III after 24 h and disappeared at 48 h where the predicted final metabolite is soyasapogenol B. These sequential metabolisms take place through sugar hydrolysis which results in the formation of more hydrophobic metabolites and smaller molecules (Kang et al., 2010).

Lignan. Low lignan bioavailability is recovered in plasma in human after ingestion of lignans (Kang et al., 2010). It is interesting that lignan is easily absorbed into plasma after ingestion. The available information is for secoisolariciresinol diglucoside and its aglycone secoisolariciresinol and matairesinol. Based on the studies, at least 40% of ingested lignans are metabolized by intestinal bacteria and these metabolites can be detected in the plasma. Metabolites of lignans appearing in the human plasma after ingestion follows the sequences: (i) at 14.8 ± 5.1 h enterodiols are maximally found in plasma, (ii) at 19.7 ± 6.2 h enterolactone is maximally bioavailable, and (iii) at 8-10 h enterolignans are bioavailable. Resident time of lignan metabolites in plasma is 20.6 ± 5.9 h for enterodiols and 35.8 ± 10.6 h for enterolactone, respectively (Kang et al., 2010).

Phytosterol. Low phytosterol bioavailability is observed in human plasma after ingestion. Definite small amount (0.6-7.5%) of phytosterol is transported through gut epithelial cells *in vivo*. Chemically, phytosterol is similar to cholesterol; yet cholesterol is absorbed at much higher levels than phytosterol. This is because of side chain differences, i.e. ethyl/methyl group in C₂₄ which increases hydrophobicity but reduces absorption; and the presence of Δ_5 double bond. The similarity of absorption mechanism of phytosterol and cholesterol is that (i) they need to be in emulsion system and (ii) to be facilitated by Niemann-Pick C1 like 1 (NPC1L1) protein. Surprisingly, it is just recently acknowledged that many of bioactive compounds need to be in emulsion system to make them more bioavailable (Kang et al., 2010).

Phytate. Phytate bioavailability is low in human plasma levels after ingestion. Plasma myo-[inositol-2-H³(N)]hexakisphosphate in human after ingestion is dose-dependent and it only reaches 3-5 times higher than that of diet poor in myo-[inositol-2-H³(N)]hexakisphosphate. Further study using rat found that absorbed phytate is quickly distributed into tissues such as brain, kidneys, liver, and bone in its original dietary molecular forms. The highest level is in brain reaching 10 times compared to average of tissues (Kang et al., 2010). This is beyond

conventional nutrition believes that phytic acid and phytate is not traversed across lipid bilayer.

Sulfur compounds. Bioavailability of isothiocyanates is better than glucosinolate in the human gut. In spite of different cruciferous origins and types, isothiocyanate is always found in plasma and its metabolites in urine is consistently found as dicarbamate or mercapturic acid. It is important to note that not all glucosinolates behave similarly. Generally, heated or cooked glucosinolate is less bioavailable (1.8-43%) than raw (8.2-113); and it is quickly absorbed in the gut and quickly excreted in urine (24 h). The exceptions are from pak choi (butenyl and pentenyl isothiocyanates, 8%), garden cress (benzyl isothiocyanate, 14%), and water cress (phenylethyl isothiocyanate, 50%) compared to 100% isothiocyanate. Critical factors of the study remain: (i) individual variations (different microflora in the bowel, metabolism, and chewing ability), (ii) natural cruciferous matrices so that strongly entrapped glucosinolate in the cells will be hardly released during chewing, and (iii) types of glucosinolates (Vermeulen, 2009).

Vegetable consumption during lunchtime shows a general lag phase for excretion of mercapturic acid at 4 h after ingestion (Vermeulen, 2009). The sulfo compounds (isothiocyanate) in the body is conjugated. Raw vegetable consumption results in a fast excretion whereas cooked vegetable has longer resident time of conjugated form. Elimination for half-life of the compound is 2-4 h, which is longer than that of other study (1.8 h) (Ye et al., 2002), with excretion rate of 0.18-0.33 h⁻¹ by assuming the first order reaction.

Food source of sulfur compounds is a determinant factor in absorption in addition to processing and physiological conditions. Sulforaphane content in raw and cooked broccoli is 9.92 and 61.4 $\mu\text{mole/kg}$, respectively; and 37 and 3.4 % of them are recovered in urine in the form of sulforaphane mercapturic acids. On the other hand, 54% of benzyl isothiocyanate from garden cress is found in urine but phenylethyl isothiocyanate from watercress after chewing is 47%. When cooked watercress is administered, only 1.2-7.3% of glucosinolates is recovered; this is much lower than sulforaphane (17.2-77.7%). Monitoring dithiocarbamate in urine shows 12% recovery from boiled broccoli sprout. The recovery increased to 80% when the boiled broccoli sprout is treated with myrosinase. About 68% of allyl isothiocyanate from mustard is excreted in urine as mercapturic acid while sinigrin is present at 15% and 37% from cooked and raw cabbage¹², respectively (Vermeulen, 2009). Generally, the routes of metabolisms in the human body vary depending on the target molecules and food sources. Glucoraphanin and sulforaphane from cooked and raw broccoli peak for maximum 6 and 1.6 h, respectively (Vermeulen, 2009). The half-life clearance of sulforaphane in the human body from the aforementioned vegetables is 4.6 and 3.8 h, respectively. These are different from half-life time of mercapturic acid which is 2.4 and 2.6 h, respectively. Further investigations using human subjects show inconclusive results that particular polymorphism S-glutathione transferase (GST M1, T1 and P1) and N-acetyl transferase (NAT2) gene affect the variations (Vermeulen, 2009). It is important to view these phenomena under the holistic affecting factors.

¹² Data is calculated from : First order kinetics

k_a : intercept and slope with a residual method; k : natural log of plasma amounts plotted against time; k_e : natural log of absolute excreted amounts vs time; area under curve (concentration vs time) extrapolated using trapezoid method; lag phase is determined from empiric curve obtained; base line is not used

Phenolics. Generally, the least absorbed polyphenols are proanthocyanidins, galloylated tea catechins, and anthocyanins (Epriliati, 2008 and Ginjom, 2009).

Caffeine. Using pharmacological principles, absorption simulations of pure compound in intestine is mimicked by caco-2 monolayers. During the simulated transit method (model A), unchanged caffeine was transported across epithelial cells (Figure 3). This indicates that caffeine is directly transported to the basolateral compartment without damaging the tight junctions. This transport is selectively occurring in the apical to basolateral direction over the bioassay time (240 min). The apical caffeine levels from simulation of transit method even after 120 min (Figure 3, top panel) are higher than that of semi dynamic apical solution method (B model) (Figure 3, middle panel). Caffeine was transported by the enterocytes in the apical to basolateral direction apparently without an equilibrium state being generated. Uptake of caffeine was rapid and basolateral secretion possibly required a certain amount of caffeine intracellularly is retained. When a high gradient concentration was maintained, continuous basolateral secretion of caffeine took place at a constant rate. As a result, the final level of basolateral caffeine was higher than the apical levels, even when it was subjected to a 22 h bioassay (C model) (Figure 3, bottom panel). The transport mechanism of caffeine may be a simple passive diffusion. However, another study shows caffeine can also be transported by the transcellular route (Mao, 2007). In addition, caffeine is found interacts with glucose uptake sensitivity (Pizziol, 1998).

Catechin. The simulation transit method of catechin indicates that it is retained in the apical compartment at about one-third of the initial amount (86.8 nmol) and remains at about the same level throughout the experiment (Figure 4, top panel). However, basolateral compartment analysis did not indicate equal amount of translocated catechin. In contrast, most basolateral samples contain very little catechin. In the static apical solution method (Figures 4, middle and bottom panels), apical catechin was reduced to 39 nmol after 22 h, but there were no indication of transported catechin in the basolateral compartment. In the present study, there may have been some metabolism of catechin based on apical losses which require further study to identify possible metabolites of catechin.

Lycopene. Lycopene is neither transported (Figure 5) nor chemically changed during bioassay using all three bioassay methods for all time periods. Its hydrophobicity and unfavorable molecular geometry apparently prevents lycopene from passing through monolayers via either paracellular or transcellular routes. This was confirmed by the decrease of TEER values for all monolayers, which is not accompanied by lycopene translocation into the basolateral compartment from the apical solutions. In the present study, the apical lycopene do not show disappearance in the transit model (Figure 5, top panel). Instead, lycopene shows apical accumulations with renewal solutions. Similar results are obtained from the semi dynamic model (Figure 5, middle panel) and confirmed in the 22 h static model (Figure 5, bottom panel). Lycopene absorption has been shown to be affected by the presence of other carotenoids, the lipid status, and plasma antioxidant capacity (Bohm & Bitsch, 1999). However, another study found that lycopene plasma levels after consumption of cherry tomatoes are insignificantly different from the plasma base line (Bugianesi et al., 2004). Further absorption from micelles has been shown to be slow (e.g. lycopene absorbed by LNCaP and Hs888Lu cells took approximately 10 h; Xu et al., 1999). This suggests that epithelial cells may have specific mechanisms that are not micelle dependent.

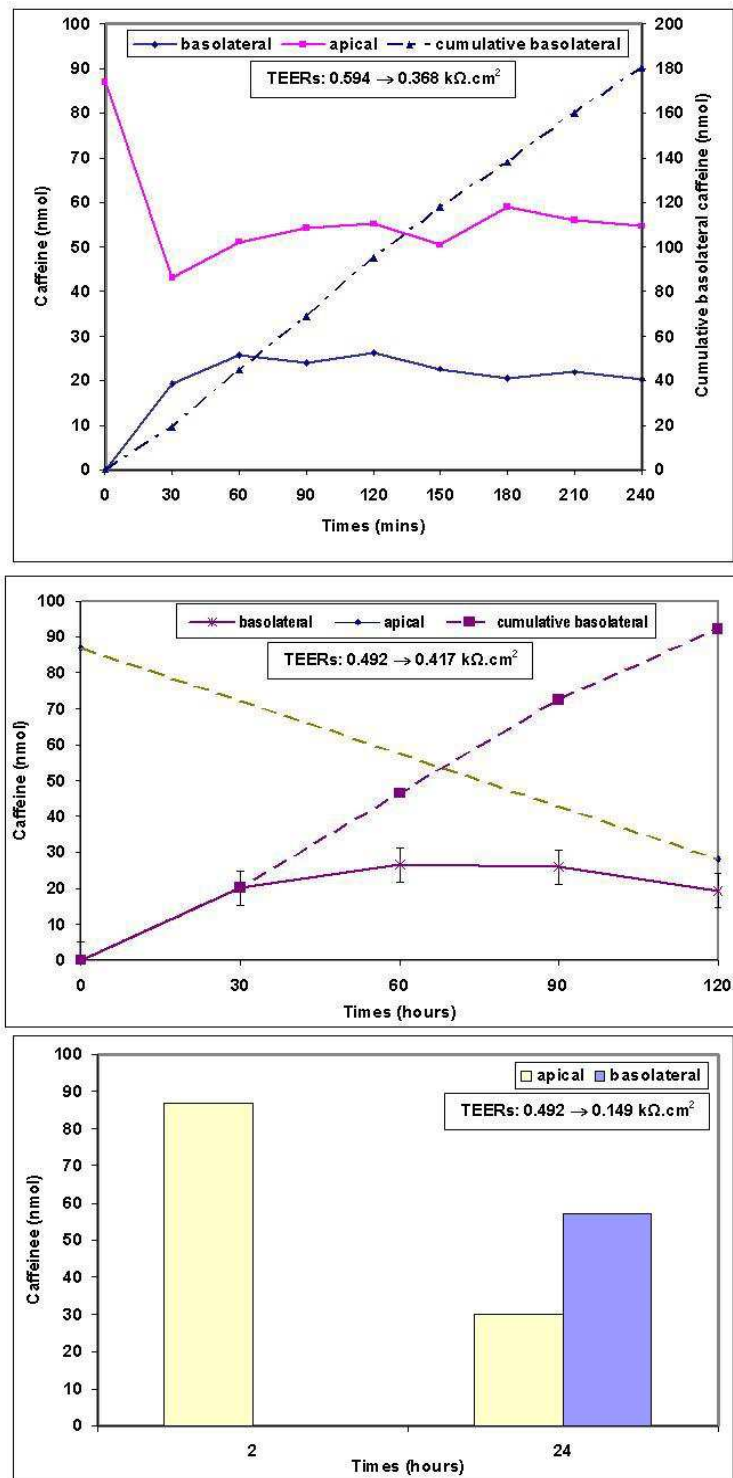


Fig. 3. Bioassay of caffeine using simulated transit method (top panel: model A; using 2 transwell-inserts), static apical solution method (middle panel: model B; using 4 transwell-inserts), and static apical and basolateral solution procedures for 22 h (bottom panel: model C; using 2 transwell-inserts)¹³

¹³ Model A: apical side is replenished every 30 min, Model B: basolateral side is refreshed every 30 min, Model C: both apical and basolateral are not replenished for 22 h

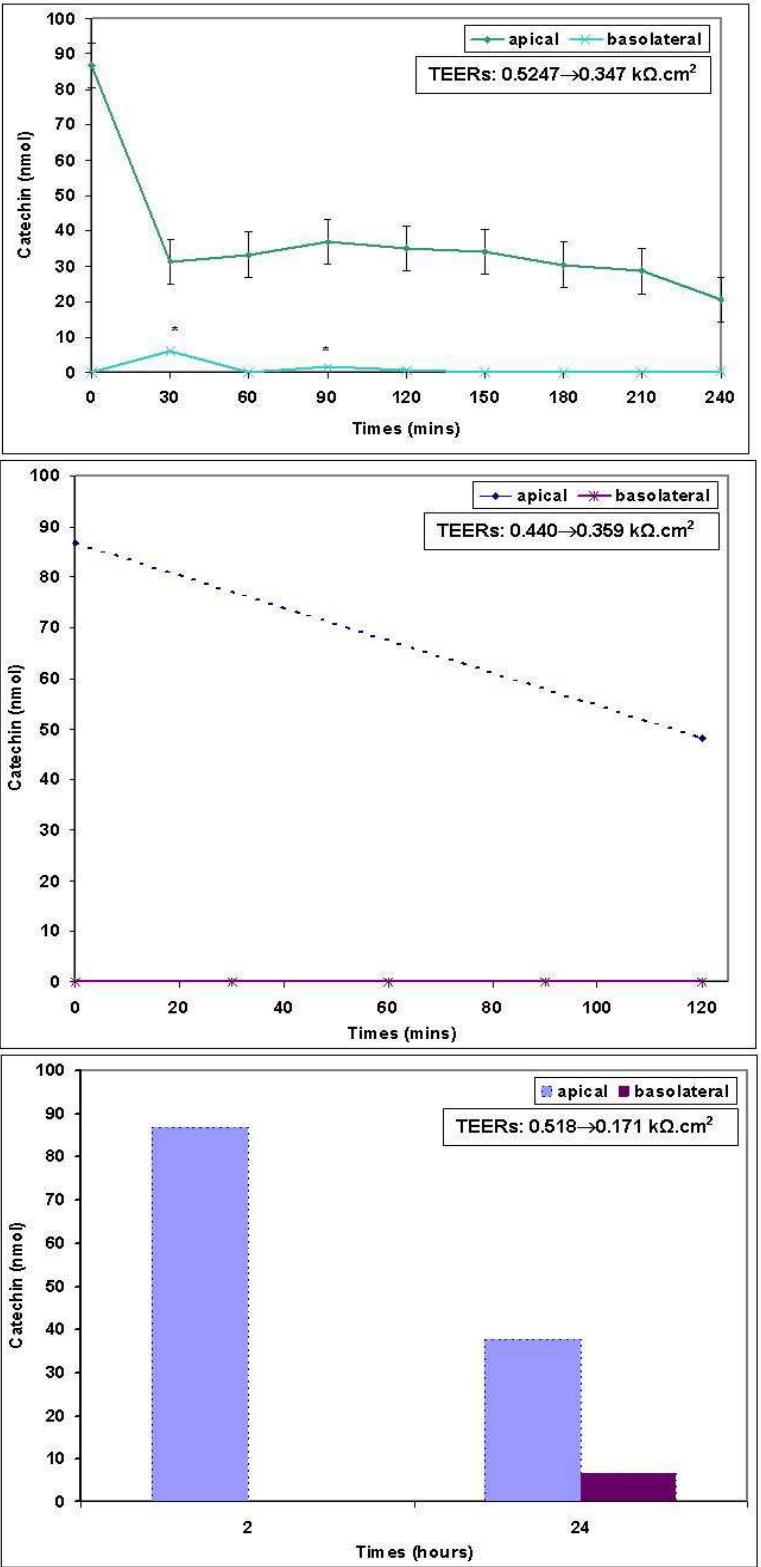


Fig. 4. (+) Catechin transport using simulation of transit chyme (top panel: model A; using 2 transwell inserts), static apical solution methods (middle panel: model B; using 4 transwell inserts), and static apical and basolateral solution after 22 h (bottom panel: model C; using 4 transwell inserts)

β-carotene. There are always reductions of apical levels but not necessarily accompanied by release into the basolateral side (Figure 6). Meanwhile, β-carotene completely disappears in the 22 h static model, both from the apical and basolateral sides although TEER values drops from 0.497 to 0.125 kΩ.cm². β-carotene may diffuse better than lycopene, as indicated by the β-carotene apical disappearances; however, neither is translocated. This may be related to intrinsic solubility, as β-carotene is more soluble than lycopene in the mixed aqueous/organic solvents. In the semi dynamic model after 120 min, apical β-carotene decreases and in the static model after 22 h, β-carotene disappears completely.

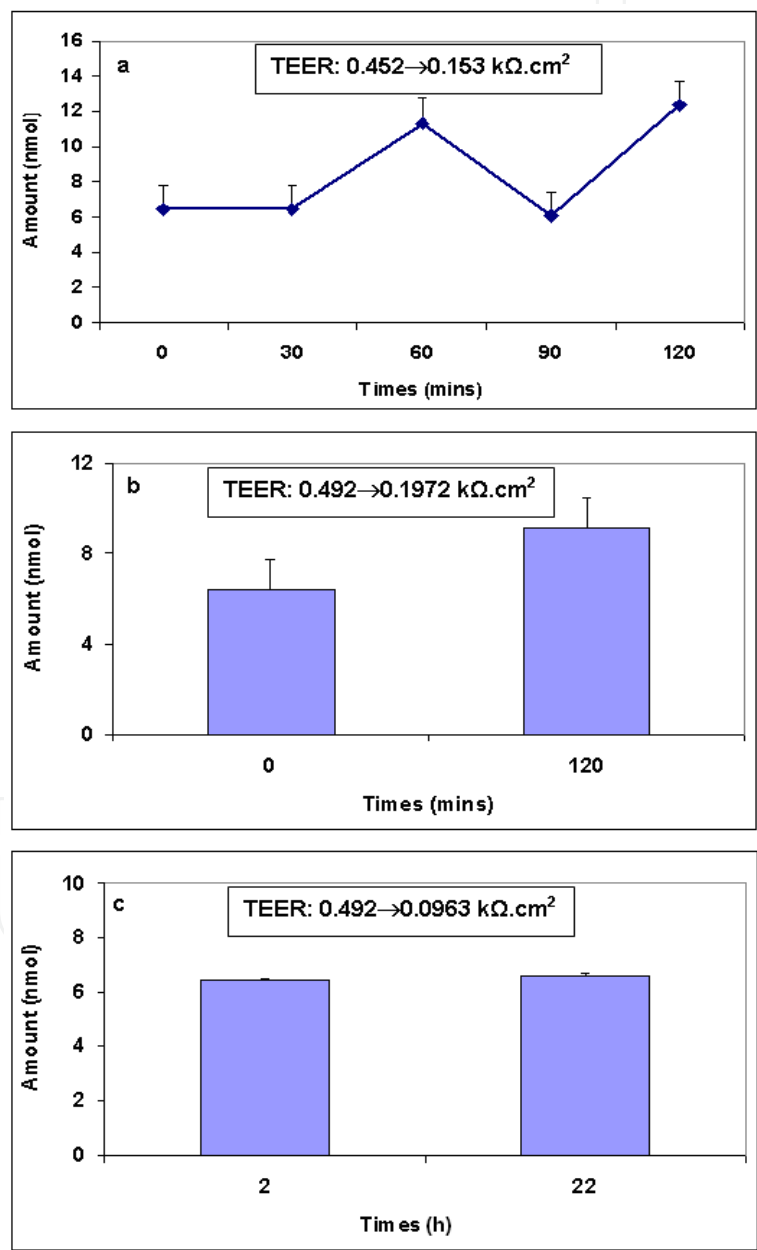


Fig. 5. Apical lycopene bioassay; **a** transit model (model A), **b** basolateral renewals (model B), **c** static model (model C) in buffer-0.5% DMSO

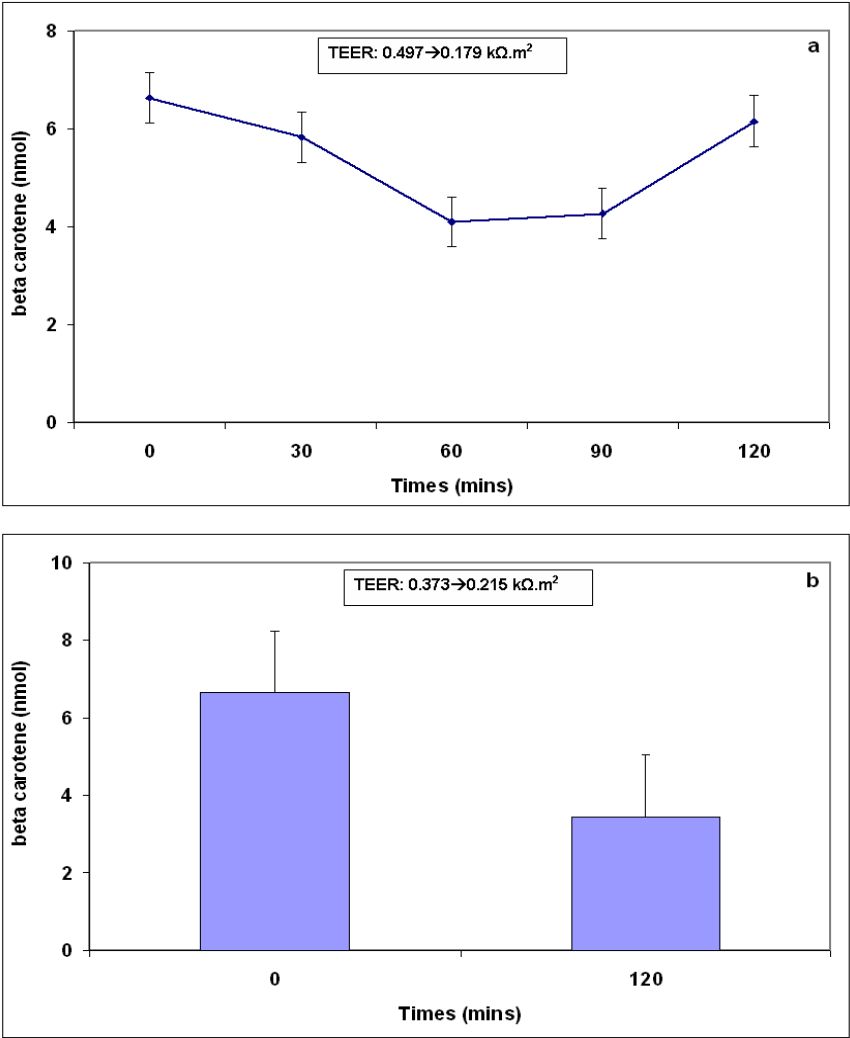


Fig. 6. Apical β -Carotene in HBSS-25 mM HEPES contained 0.5 % DMSO: **a** transit chyme (model A), **b** static apical solution (model B)

4.3 Dosages

Establishing the most suitable dosages for an optimal health benefit of a phytochemical is not an easy task. As an antioxidant, phytochemicals are generally required in small doses due to its ability to become pro-oxidant. Based on its traditional usage, the doses are commonly determined from folklores, thus the key compounds mostly responsible for their health functions and their mechanisms remain to be explored through epidemiological studies. Table 3 lists what doses studied *in vitro* and folklores.

Phytochemicals	Effects	Dosages
Soya saponin (Kang et al., 2010)	<ul style="list-style-type: none">• Inhibits metastasis HT-1080 cells• Decrease HT cell growth• Inhibit AFB₁-DNA adduct formation in HepG2 liver cells• Induce apoptosis in SNB 19 glioblastoma cells	<ul style="list-style-type: none">• 100-300 μl/ mL; 24 h• 150, 300, 600 ppm; 72 h• IC₅₀ at 30 μg/mL; 48 h• 25-75 μM; 48 h

Phytochemicals	Effects	Dosages
	<ul style="list-style-type: none">• Suppress HTC 15 cell proliferation• Induce macroautophagy• Decrease migratory ability/increase adhesion of B16F10 cells• Enhance adhesion of MCF7 cells	<ul style="list-style-type: none">• 25-500 ppm• 100 ppm; 24 h• 25-75 µM; 12 h• 50 µM; 24 h
Soyasapogenol A and B (Kang et al., 2010)	<ul style="list-style-type: none">• Suppress HT 29 cell growth	<ul style="list-style-type: none">• 6-50 ppm; 72 h
Soyasaponin – soyasapogenol B monoglucuronide mixture (Kang et al., 2010)	<ul style="list-style-type: none">• Suppress HT 29 cell growth	<ul style="list-style-type: none">• 50 ppm; 72 h
Phytate (Kang et al., 2010)	<ul style="list-style-type: none">• Decrease expression of TNF-α and TNF II in Caco-2 cells• Inhibit proliferation of HT 29 cells• Inhibit growth of MCF-7/Adr cells• Inhibit growth of MDA-MB 231cells• Inhibit growth of MCF-7 cells• Inhibit growth of HepG2 cells• Inhibit growth of LNCaP cells• Inhibit growth of DU145 cells	<ul style="list-style-type: none">• 1, 2.5, and 5 mM; 12 h• 13 mmol.L; 12 h• IC₅₀ at 1.26 mM; 96 h• IC₅₀ at 1.32 mM; 96 h• IC₅₀ at 4.18 mM; 96 h• 0.25-5 mM; 6 d• 0.5-4 mM; 24 h• 0.25-2 mM; 24 h
Phenolics in colourful potatoes	<ul style="list-style-type: none">• Treating gastric ulcer	<ul style="list-style-type: none">• 2-3 times a day for no more than 4-6 weeks; drinking diluted water extract of fresh potato (Tan & Rahardja, 2010)
Phytochemicals from luffa [<i>Luffa cylindrica</i> Roem.; <i>Luffa Aegyptica</i> Mill.; <i>L. Cattupincina</i> Ser.; <i>L. Pentandra</i> Roxb.]	<ul style="list-style-type: none">• To treat intestinal inflammation• To improve breast milk production• To treat asthma	<ul style="list-style-type: none">• 1-2 times a day; drinking mature seed extract made from 20 g powder in a ½ cup of hot water• Drinking extract of a 6 g of seed and flesh powder• 2-3 times a day; drinking young luffa juice sweetened with sugar
Phytochemicals from Indian champor weed [<i>Pluchea indica</i> (L.)	<ul style="list-style-type: none">• For fever and emetic sweat removal• For body odour removal	<ul style="list-style-type: none">• once a day; consuming 10 g of boiled leaves• regularly 3 times a day; consuming 10-15 pieces of

Phytochemicals	Effects	Dosages
Less.]	<ul style="list-style-type: none">• For relieving gastrointestinal disorders in children	<ul style="list-style-type: none">raw or steamed leaves with rice• Consuming 3-5 pieces of crushed leaves mixed with soft rice (porridge) (Dalimartha, 2005; Hariana, 2006).
Phytochemicals from Watercress [<i>Nasturtium officinale</i> R. Brown, <i>N. officinale</i> W.T. Aiton, <i>N. nasturtium-aquaticum</i> (L) H. Karst., <i>Radicula nasturtium</i> Cav.,]	<ul style="list-style-type: none">• For tuberculosis• For inflamed lung and coughing• For skin irritation• For urinary problems	<ul style="list-style-type: none">• 2-3 times a day; consuming soup made from 250 g watercress and pig bone, added with sufficient salt• Consuming soup made from 60 g of watercress and sugar• several times a day; consuming soup of boiled watercress• Consuming soup made from 250 g of watercress and palm sugar (Muchlisah & Hening, 2009)
Phytochemicals from bilimbi [<i>Averrhoa bilimbi</i> Linn]	<ul style="list-style-type: none">• For hypertension• For acne• For muscle pain• For teeth cavities	<ul style="list-style-type: none">• once every three days; drinking extract of 3 bilimbi fruits in 3 glasses of water, concentrated 3 times• 3 times a day; applying a mixture of 6-8 ground bilimbi fruits, a ½ tea spoon of salt, a ¼ glasses of water onto acne• Applying a mixture of 25 pieces of bilimbi leaves, 10 clove, and 15 pepper, ground finely, added with a small amount of vinegar, at suffering body/tissues• Chewing 5 pieces of bilimbi fruits with a little salt and at the suffering teeth (Hariana, 2006).
Phytochemicals from Glossy nightshade, Black nightshade [<i>Solanum americanum</i>	<ul style="list-style-type: none">• For urethra infection	<ul style="list-style-type: none">• twice a day; drinking a ½ glasses of extract of 30 g of black nightshade fruits with <i>Hedyotis diffusa</i> grass, and <i>Phyllantus urinaria</i> in a 3 glasses of water,

Phytochemicals	Effects	Dosages
Miller, <i>Solanum nodiflorum</i> Jacq, <i>Solanum nigrum</i> auct non L.]	<ul style="list-style-type: none">• For eczema or dermatitis• For xerophtalia• For cervical erosion• For pektay	<p>concentrated twice</p> <ul style="list-style-type: none">• twice a day; consuming 60 g of boiled shrub• 3 times a day; chewing around 15 black nightshade fruits• 1-2 times a week for 8 weeks; applying a mixture of ground boiled-black nightshade fruits at the suffering tissues• 3 times twice a week; drinking a ½ glasses of decoction of 30 g ground black nightshade fruits and <i>Celosia cristata</i> flowers in a 3 glasses of water, and concentrated (Dalimartha, 2008)
Phytochemicals from Waxy gourd [Benincasa hispida (Thunb) Cogn., B. cerifera savi, Cucurbita hispida Thunb. Lagenaria dasystemon Miq]	<ul style="list-style-type: none">• Curing hemorrhoid• To treat diabetes	<ul style="list-style-type: none">• once a day for 14 days for adult; given infusion liquid of 1 waxy gourd fruit as big as a palm hand, added by 10 pieces of anises/fennels, a ±1cm length of <i>Alixa stellata</i>, and a tea spoon of honey; (Kementerian Lingkungan Hidup, 2011)• Consuming 100-150 g boiled or juiced waxy guard (Wijayakusuma, 2008)
Phytochemicals from Lemon basil [Ocimum americanum, O. citriodorum, O. africanum, O. canum Sims, O. brachiatum Blume]	<ul style="list-style-type: none">• To ease people suffering from early ejaculation, late menstruation, breast milk and gas cleanser in the human body, and for removing fever	<ul style="list-style-type: none">• twice a day; drinking a ½ glasses of decoction of 15 g lemon basil grass in a 2 glass of water for 15 minutes (Hariana, 2006)

Table 2. Resume of dosages used in studies regarding phytochemicals health effects and in folklores¹⁴.

¹⁴ The information of Indonesian medicinal folklores is obtained through a collaboration project between Korean Food Research Institution and Bogor Agricultural University, Indonesia in 2011.

5. Recommended daily allowance

5.1 Recommended daily allowance

Recommended uses and maximum limits of uses in modern public health management are limited. The ancient uses are based on folklores and old documents. This information should be followed up with proper scientific investigations and documentations. Even for broccoli which is extensively studied, the recommended daily intake has not been officially established. US national cholesterol education program recommends adult subject to consume 2 g of phytosterol/d for optimally lowering LDL-C and coronary heart disease risks by 10% (Kang et al., 2010). The mechanism of this is still vague but it is known that phytosterol/phytostanol does not necessarily present simultaneously with cholesterol to control cholesterol absorption.

5.2 Public health management

There is limited information on detailed diet prescription aiming at treating a particular disease, except those recorded in ancient medications. Dieticians usually arrange diets for patients not aiming for disease treatments but to meet certain nutritional requirements to improve their stamina or immune system to combat their physiological problems. Mechanism for phytochemical health benefits have been studied extensively. Current understanding shows that public health would take benefits from diet management for prevention and maintaining public health instead of treating it. Many research results found scientific base of phytochemicals. For example, liminoids has increasingly proven positive health effects including induction of glutathione S-transferase, inhibiting cancers growth, and lowering cholesterols (Kang et al., 2010), yet officially, this still has not been established for recommended daily allowance. On the other hand, information from ancient medicinal prescriptions as listed in Table 3 is mostly in the form of decoction of the phytochemical sources and the boiled water is drunk. Interesting research area is to establish whether such preparation preserve biological functions of the phytochemicals or, instead, the methods modify molecular form of the phytochemicals that is a much safer and/or more bioactive than its original forms.

5.3 Phytochemicals incorporation in diets

Phytochemicals are commonly consumed as supplements either in capsules, tablets, or powders. The incorporation of such ingredients in food products may or may not face problems of stability, especially at its extraction step and formulation and food processing in which heating is one of predominant aspects for generating food palatability. Most conventional food preparations are of high risks on phytochemical instability. Attempt to improve food technology remains inconclusive. Health effect study indicates that enriched ground beef with soy phytosterol reduces total cholesterol, LDL-C, and TC/HDL cholesterol by 9.3, 14.6, and 9.1%, respectively (Vermeulen, 2009). Such attempts require standardization for establishment of functional food regulations.

5.4 Phytochemical demands per capita

In order to maintain health where phytochemicals are involved, a daily recommended allowance similar to other nutrients is required. Therefore, prior to daily recommended

allowance establishment, there is a need for dosage allowance for each bioactive. Similarly, when recommended allowance has been established, food chain supply needs to provide necessary quantity of the phytochemical sources for people. Such data are currently unavailable, and thus, a database and information system for it needs to be established.

6. Conclusion

Phytochemicals bioavailability is strongly dependent on cell wall compositions of the food matrices they originate from, structural chemistry of the phytochemicals, history of processing, as well as individual human gastrointestinal system. Determination of phytochemical bioavailability is increasingly developed using both *in vitro* and *in vivo* approaches, and yet the results are still inconclusive. The main challenge is to develop an *in vitro* model that can represent human *in vivo* condition for practical uses. On the other hand, many aspects of bioavailability is not well understood, prompting further research and database for recommended dosages and consequently per capita phytochemical demands for public health management. Currently, folklores are the main sources of public health management using phytochemicals and database remains to be pursued for better scientific base of folklores practices.

7. References

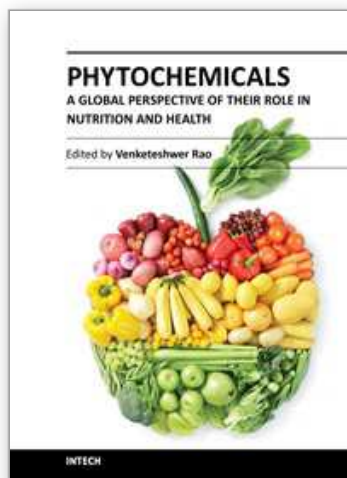
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