JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Nutriomic Analysis of Fresh and Processed Fruit Products. 1. During in Vitro Digestions

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Nutriomic analysis is a postgenomic-based study of nutritious components (nutriome). There is a need for an in vitro digestion and absorption model to unravel interactive factors varying nutriome release from various food materials that cannot be directly studied in humans. Effects of processing and in vitro digestion steps on carotenoid, sugar, and organic acid release from tomato, papaya, and mango products were comprehensively studied for the first time in this research. In vivo chewing experiments using 24 healthy adult volunteers was carried out prior to chewing simulation. Microscopy showed that cutting and blending alone were unlikely to mimic chewing at swallowing point. Using general linear model (GLM) ANOVA and principal component analysis (PCA), effects of interaction between digestion steps and processing types on the nutriome release were significant ($p < 0.05$) when 90% particles of 0.5 (dried) and 1.5 cm (fresh) were digested in vitro. Generally, dried and fresh fruits released lower levels of nutriome components than juices. PCA indicated nutriome release from tomato products was affected by the factors studied more than those from papaya and mango products. Fruit type is the main determinant factor relative to processing and digestion steps because it determines the extent of matrix that breaks down and consequent nutriome diffusion rates. It is predicted that pectin plays a role in determining the rate of nutriome release and absorption, which requires further investigation.

KEYWORDS: Nutriomic analysis; PCA; fresh and processed fruits; in vitro digestion; tomato; mango; papaya

INTRODUCTION

Doring (*1*) defined a study of the nutritious components of foods and their relationships to activities of the individual biology of humans (e.g., digestion and uptake, health) as **nutriomics**. For optimal biological functions, it is important to consider the total complement of nutritional molecules (nutriome), as there are a large number of potential interactions of food components with the human biology, particularly at the level of gene transcription and regulation. Hence, nutriomic analysis aims to discern the roles of nutriome components in the human digestive system, comprehensively.

Genetically, health is an equilibrium state of healthy−unhealthy conditions, called epigenetic (*2*) illustrating the complexity of the interactions and providing a schematic framework for the assessment of the efficacy of food nutriomes. Mechanistic understanding of functional ingredients acting in the human body to gain a healthy net resultant in epigenetic driven condition is still not well established. First, to exert its functions in human health certain levels of bioavailability of the beneficial components in the human body are required. This is currently beyond nutrient contents in the foods and residual levels of metabolites in urine or feces, but in vivo studies with human subjects are unlikely. Moreover, surrogate models from animals may have no physiological similarities. Therefore, assessing the claim of the efficacy is not an easy task, mainly due to lack of the many systematic data required. Extensive research is required from many disciplines comprehensively, though, and an in vitro model mimicking the human network system is necessarily available for routine claim assessment in functional food manufacturing.

Although enzyme and bile salt concentrations and reaction conditions of in vitro digestion have been well studied for single-nutrient bioavailability determinations, less attention has been paid to the beginning (chewing) and end (uptake) of the human digestive process; for example, models do not involve a chewing step (*3*−*9*); powders or pulverized solid samples are used (*3*−*9*); extracts of bioactive compounds are studied (*10*); or mincing is performed (*11*). In addition, absorptive tissues were mimicked using dialysis tubes (*12*−*14*) or cell monolayers (*3*−*10*) or referred to animal in vivo studies (*15, 16*). Cell monolayers are the most important potential model because physiological characteristics of epithelial brush borders are expressed even though at lower levels (*17*−*23*).

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10.1021/jf900368p CCC: $40.75
Published on Web 03/16/2009

The novelty of the present nutriomics analysis involves (a) the use of simulated chewed particles and the use of Caco-2 cell monolayers as a model of apical−basolateral transport that mimics membrane modeling of the intestinal human brush border in delivering nutriome from the intestinal lumen to the circulatory portal entrance, in addition to the conventional biochemical mimics of mouth, stomach, and intestinal environments and (b) the use of principal component analysis (PCA) to map detectable nutriome components and their interactions. The first paper in this pair investigated in vitro digestion of minimally processed fruits and vegetables, mapping the possible interactions of nutriome components using PCA.

Fruits and vegetables are frequently consumed as minimally processed solid products, the tissues and cell walls of which may be undisrupted. Here, the nutrients are located in cells encased by cell walls composing the food matrices at the macro level. Such natural organizations may limit nutrient bioavailability along the gastrointestinal tract, and processing is expected to modify bioavailability (*24*). It is evident that cell rupturing in ingested foods is crucial for nutrient bioavailability of processed and unprocessed samples (*25*) in vitro and in vivo (*26*), respectively. Furthermore, the activity of in vitro pepsin or pancreatin does not solubilize or degrade cell wall components of spinach (*27*). Thus, nutrient bioavailability from plant foods may ultimately depend on particle characteristics after the chewing process.

The importance of chewing can be seen from the reported reduction of plasma glucose level when participants swallowed without chewing samples of diced potato or apple, whole rice, or sweet corn (*28*). The effects of chewing on important tropical fruit such as fully ripe mango papaya and on their products, which are known to affect diabetes patients (*29, 30*), have not been described systematically. Thus, the physical changes of particles during in vivo chewing and the rheological consequence for swallowing were investigated. The in vivo chewing products were then simulated for the in vitro digestion model.

Chewing of solid foods is a complex process governed by neurophysiology mechanisms (*31, 32*), which generate new surface areas and produce a food bolus, a mixture of chewed particles and saliva. The final products of chewing vary depending on the food types and texture of the ingested foods, with this process being compounded by differences in the chewing ability of individual humans (*33*). At the end of chewing, particles can range from micrometers to 0.5 cm size (*34*−*37*); however, particles with 1.5 cm length or more have been obtained from the chewing of pasta and bread (*11*). In addition, effects of texture on chewing have been shown; for example, tender meat was comminuted better than tough meat boli during chewing (*38*). Compactness and agglomerations also occur during chewing (*36, 37*). However, the effects of chewing on in vitro bioavailability have not yet been studied systematically.

Studies of bioavailability using powders and diced, pureed, or blended samples may lead to less realistic bioavailability values, especially when natural foods such as fruits or vegetables are studied. A chewing study is more realistic when heterogeneous natural foods are used instead of synthetic samples (*39*); thus, in vitro bioavailability determinations based on such study are also probably more realistic. In vitro models have been widely applied in nutrient bioavailability studies, for example, grape extract (*10*), rice, maize, wheat, fish, carrot, infant formula, iron, and its interactions with other food components by Glahn and colleagues (*3*−*7, 40*−*48*).

Tomato, mango, and papaya are fruits containing bioactive compounds, pectin-rich cell walls, and high levels of sugars. These components play roles individually in human well-being, but excessive levels may counter health benefits (e.g., excessive sugars can cause diabetes, obesity). It is a major challenge to determine how much of each of those compounds is delivered (potential bioavailability) to the human circulatory system (bioavailable) from their consumption in particular amounts and to identify their subsequent effects on human health. Information on the changes to food components by the human digestive system may help explain relationships between diet and human health that are currently inferred from epidemiology studies.

Besides 9-*cis*-β-carotene, papaya and mango are rich in xanthophylls, neoxanthin, violaxanthin, lutein, zeaxanthin, and cryptoxanthin (*49*). Currently, the carotenoid contents of mango and papaya have been reported, but little information on the health effects of carotenoids through papaya or mango consumption has been reported.

The present study is the first attempt to test the nutriomic digestion approach for a range of common fruit product types (fresh, juiced, dried) used in daily life. Using simple processing such as juicing and drying, without any additional ingredients, nutriomic analysis of tomato, mango, and papaya involving in vitro digestion was investigated. Whereas the selected fruits have different textures and biological architecture, they share common components such as carotenoids, sugars, and organic acids, which are believed to affect human health. Therefore, a comparison among different fruits and their products, based on observations for the same compound groups, was carried out.

MATERIALS AND METHODS

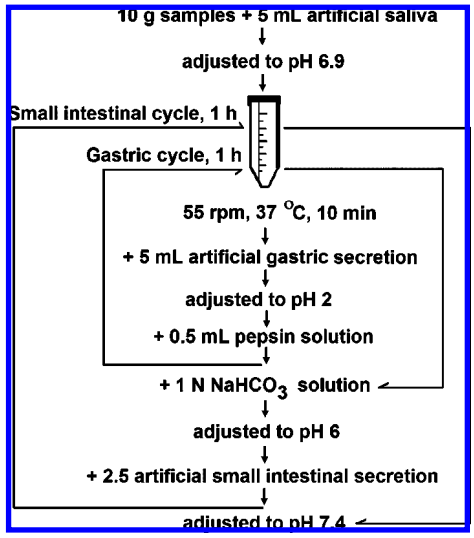
Materials. *Digestion Samples.* Fresh samples of Kent mango (*Mangifera indica*) (supplied to market from Bowen, northern Queensland, Australia), Red papaya (*Carica papaya*), and Red Roma tomato (*Lycopersicum esculentum*) were purchased from a local store in Brisbane, Australia. All fruits are fully ripe fruits ready to eat as table fruits.

Chemicals included piperazine-*N*,*N*′-bis[2-ethanesulfonic acid] (PIPES), casein, amylase (human salivary amylase IX), pepsin, porcine pancreatin, porcine bile extract, buffer phosphate pH 7.0 HPCE (high-performance capillary electrophoresis; to help with nutriome component mineral), caffeine, caffeic acid, *p*-coumaric acid, *o*-coumaric acid, mangiferin, chlorogenic acid, ferulic acid, fumaric acid, naringenin, hydroxy benzeoic acid, (+)-catechin, succinic acid, *trans*-aconitic acid, malonic acid, malic acid, citric acid, oxalic acid, L-ascorbic acid, tartaric acid, lycopene, β-carotene, Dowex 1x8 chloride forms 100−200 mesh, Dowex 50Wx8 hydrogen forms 100−200 mesh, and Chelex 100 (Bio-Rad); lutein and β-cryptoxanthin (Extrasynthese); Optivin enzyme mix, that is, a mixture of pectinase, hemicellulase, cellulase, and protease (Enzyme Solution Pty, Ltd.); nitrocellulose filter membranes 0.45 μm and nylon filter membranes 0.45 and 0.2 μm, plastic syringe 1−10 mL, and SePack solid-phase extraction cartridges (Phenomenex); weak cation exchange (WCX)-Oasis cartridges (Waters); 50 mL Falcon tubes and C_{18} powder (Alltech).

Methods. *Sample Preparations.* For each batch experiment, samples of 36 mangoes (3 boxes of 12) were peeled and chopped, and mango flesh from one mango was divided into three groups for fresh fruit, juice, and dried fruit. Similarly, 3 packs of 750 g red tomato and 3 red papayas were prepared for fresh, juiced, and dried samples. Digestions were carried out simultaneously for all fruit products in each batch of experiment containing all treatments and controls (instead of fruit samples, aquadest was digested). Therefore, fresh and juiced fruits were stored at −20 °C for 2 days waiting for dried samples.

Dried products were produced without additional ingredients using an Incubator IM550 with a microprocessor temperature controller (Crown Scientific) and a forced-fan air circulation, set at a temperature of 50 °C for 2 days. Fresh samples having initial dimensions of

Scheme 1. In Vitro Digestion Protocol



approximately 4−9 cm (L) × 3 cm (W) × 1 cm (H) were dried. All products experienced storage at −20 °C before experiments or analyses. Sample moisture contents were determined gravimetrically using a vacuum oven at 70 °C (−60 kPa) until constant weight was obtained. Fruit juices were prepared using a Juice Fountain (Breville JE900) filtered through cotton wool and were not pasteurized.

Chewing Experiments. The chewing experiment was approved by the Human Ethics Committee at The University of Queensland (Ethical Clearance No. 2005000740), and signed consent forms were completed by the participants. Here, 23−24 healthy volunteers (24−54 years old) were asked to chew 300 g (fresh) and 10−25 g (dried) samples. Participants spat out the chewed samples when they "desired to swallow". Chewed materials were weighed immediately after participants finished chewing and were mixed with 70% ethanol to keep them clean and to prevent unexpected biochemical changes and then sorted with a set of Australian standard sieves (75, 125, 250, 500, and 710 μm; 1.4, 2.36, and 4 mm) combined with sieves (homemade) consisting of industrial standard wires with apertures of 1.1 and 1.7 cm for dried fruits. Samples that could not be analyzed on the same day as the chewing were stored at 4 °C in closed containers for no more than 48 h. Water (2−5 L) was used to flush the chewed samples through the sieves; this also forced clumps of particles into individual particles. Water was preferred to vibration as clumpy chewed material was inefficiently separated by vibrations. The retained particles were drained (for approximately 5 min) and photographed, and particles were gathered and weighed.

Chewed Material Simulation. A previous chewing study showed that it is important to mimic the simultaneous punch and gentle squash action of teeth. Therefore, the solid samples were hammered gently once with a grooved food hammer (the hammer weight was 173.77 g, the grooves were 2 mm^2 with pyramid shapes) that was dropped from 2.5 cm above the sample surface (estimated as normal opening mouth under mouthful chewing). Thus, the simulated particles for human chewing contained expressed juices, due to hammering, as well as blended particles and coarse particles (0.5−1.5 cm). To limit cell wall barriers, the fruit juices were used.

In Vitro Digestion. Detail developed in the in vitro digestion model was justified on the basis of the literature (**Scheme 1**), and compositions of artificial gastric and small intestinal juices were as described in **Table 1**. In vitro digestion conditions were based on an average daily consumption of fruits and vegetables of around 375 g (reported range of 75−675 g for children (4 years old)−adult (+60 years old) (*51*)) and total fluid from ingested foods/drinks and secretions in gastrointestinal tract of 9 L a day (*52*); thus, a ratio of total artificial secretions to dry matter of samples of 1 mL:25 mg or 10 mL:0.25 g was attained during each meal time. However, products studied were juiced, dried, and fresh fruits, where the results will be compared. For a better comparison basis for dried fruit samples, moisture contents were adjusted to fresh samples (80−95%). To help with detectable levels of phytochemicals in digest solution, concentrating the digest was considered. Hence, to start with in vitro digestion, a ratio of 10 mL:1 g was used at chewing mimic.

When boli enter the gastric, there must be already gastric secretion mimicking an empty stomach; thus, an additional 5 mL of gastric juices was added. Samples were incubated in a shaking water bath at 37 °C and 55 rpm consecutively from chewing, gastric, intestinal, and gastric-intestinal steps.

Gastric digest solutions (G) were obtained through centrifugation at 4400 rpm at the end of the in vitro gastric digestion step. The residual pellets were used as "intestinal digestion only" samples (I). For "simultaneous gastric-intestinal digestion" (G+I), there was no centrifugation between the gastric and intestinal steps. Treatments were replicated three times for 27 combinations of fruit product type−digestion steps.

pH adjustments used 1 N HCl or NaOH and burette titration, and the mixtures were then vortexed. Prior experiments were used to determine the average volumes of NaOH or HCl required for pH adjustment (six replications for each sample). This accelerated the pH adjustment process. pH was measured using a calibrated digital pH-meter (TPS pH cube).

Chemical Analysis. Sample preparations were carried out according to **Scheme 2** aimed to produce a simultaneous separation method. The scheme was developed according to a simultaneous analysis for wood metabolic profiling (*53*). Extractions with wet samples were preferred to snap original chemical compositions as soon as possible, such as those applied in metabolomic. When it was possible, HPLC methods were kept using acetonitrile-based solvent for a universal mobile phase composition. Validation was carried out for standard compound solutions both undigested and digested in vitro showing recoveries as follows: 98.0% (sucrose), 86.3% (glucose) and 81.4% (fructose); 60.4% (ascorbic acid) and 109.4% (citric acid); and 65% (catechin).

Sugars HPLC-ELSD. This was run in an Agilent 1100 coupled with an evaporative light scattering detector (ELSD) 2000. The clean aqueous fractions were eluted through a 250 × 4.6 mm 5 μm Prevail Carbohydrate ES column (Alltech) with isocratic elution with 75% acetonitrile/water at a flow rate of 1 mL/min. In the ELSD, solutes were nebulized with N_2 gas at a 2 L/min flow rate and solvent was evaporated at 65 °C. The impactor, which functioned to remove the finely nebulized particles, was turned off because no more salt interferences existed in the samples. The impactor works as a particle size sorter (Analytical Service Laboratory, School of Land and Food Sciences, The University of Queensland).

Carotenoid LC-DAAD. The instrument used is an LC Waters Alliance 2690 machine coupled with an MS ZMD with Masslynx software (Micromass Ltd.). Chloroform extracts were eluted through a 250 × 4.6 mm 5 μm Grace Vydac C_{18} polymeric reverse phase, non-end-capped column (Grace) with MeOH/THF/water (67:27:6). The extracts were analyzed using a Waters Alliance 996 diode array detector (DAD) for better UV spectra for identifications, and compounds were detected at 200−700 nm. Reconstitution of chloroform extracts was similar to the HPLC-UV method (Analytical Service Laboratory, School of Land and Food Sciences, The University of Queensland).

Organic Acids and Vitamin C HPLC-UV. Organic acid analysis was conducted using a silica-based Prevail organic acids 250 × 4.6 mm, 5 μm column (Alltech), in an Agilent 1100 HPLC, but detected with a UV detector. Twenty-five millimolar K_2HPO_4 (A) and acetonitrile (B) mobile phase flow rate at 1 mL/min was used. The elution gradients were as follows: 0−12.50 min, 0% B; up to 16 min, 15% B; and returned to 0% B for another 12 min. The UV detector was set at a wavelength of 210 nm (Analytical Service Laboratory, School of Land and Food Sciences, The University of Queensland).

Statistical Data Analysis. Data sets of nutriome releases during in vitro digestions were analyzed with Minitab 15 in two steps: (1) ANOVA using general linear model (GLM) for three factors and three factor levels for each fruit; and (2) PCA for simultaneous pattern analysis of nutriome release from all fruits, to compare fruit types.

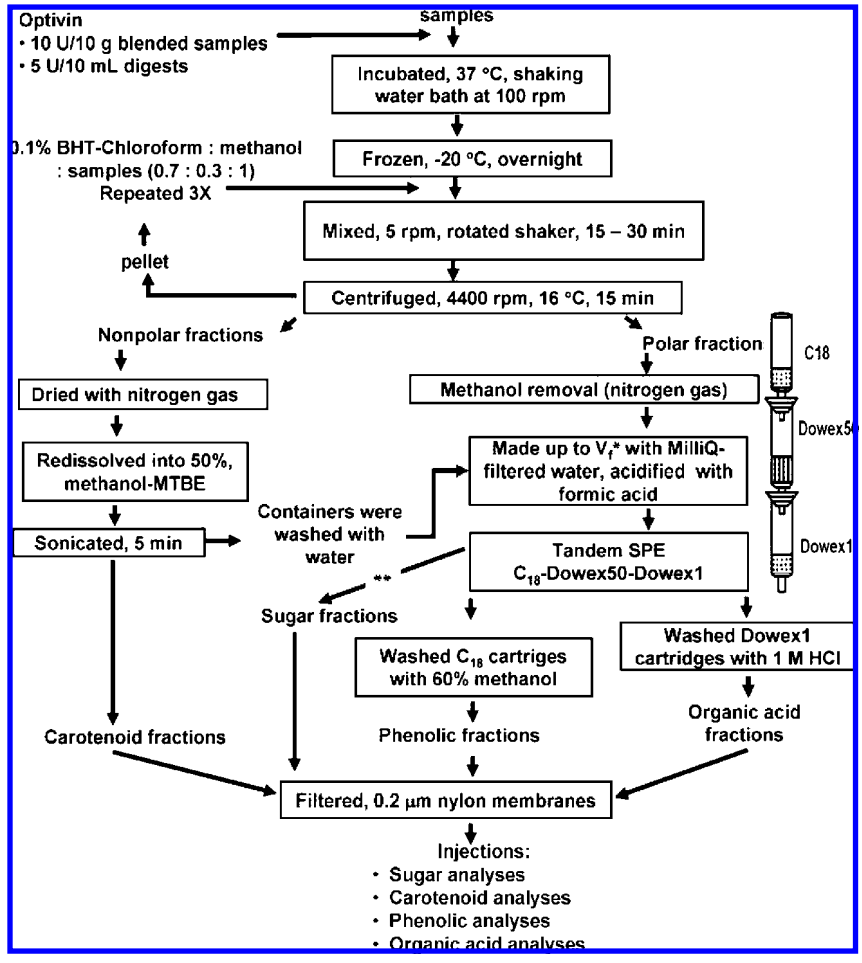
RESULTS AND DISCUSSION

In Vivo Chewing and Simulation of Chewed Particles. Primary factors affecting the highly variable chewing of food

Table 1. Chemical Composition of Artificial Gastrointestinal Juices for in Vitro Model Used in the Present Research

	mouth	gastric	intestine
pH	6.9	2	6
compositions	diluted phosphate buffer saline containing negligible α-amylase; 1.336 mM $CaCl_2$; 0.174 mM $MgSO_4$; 12.8 mM KH_2PO_4; 23.8 mM $NaHCO_3$ containing casein as a proline-rich protein(*50*); (modified from Laurent et al. (*10*)	130 mM NaCl; 5 mM PIPES; 5 mM KCl; 2000−62500 U porcine pepsin/mL in 0.1 N HCl(*3, 43*)	7.41 USP porcine pancreatin; 11.11 mg of bile extract/mL 0.1 N $NaHCO_3$
sample/gastrointestinal juice ratios	1 g (fresh; juiced):0.5	1 g (fresh; juiced):0.5	1 g (fresh; juiced):0.25
	1 g (dried):10 [solid samples are hammered and cut into 1.5 cm cube (fresh), 0.5 mm (dried)]	1 g (dried):5; giving 1 g (fresh; juiced): 1E-3 pepsin ratios or 1 g (dried): 1E-2 pepsin	1 g (dried):2.5; giving ratios of 1 g (fresh; juiced):3.6E-4 pancreatin or 1 g (dried): 3.6E-3 pancreatin; and 1 g (fresh; juiced): 2.1E-3 bile extract or 1 g (dried): 2.1E-2 bile extract

Scheme 2. Extraction Protocol before Chromatographic Analyses (Vf* = 20–30 mL for Digested and Undigested or 0.5 mL for Bioassay Samples)



products need to be unraveled in order to incorporate a chewing model into a consistent in vitro method for bioavailability measurements. From this and previous studies, it is apparent that these factors should include food textural characteristics (*11*, *33*), human variation in the unit chewing "instrument" (*35*), and responses of the oral cavity to different food characteristics (*54*). Moisture contents of samples and their hydration capacity are listed in **Table 2**.

Chewing is a process of simultaneous cutting and compression due to teeth and oral cavity volumes. At the point at which participants wished to swallow, this produced various particle characteristics of ingested samples, that is, fine to large particles that had rough surfaces and irregular shapes (**Figures 1**−**4**). Because of irregular, unsteady, and brief jaw compression during chewing, the effects of cutting by teeth were incomplete, resulting in flattened flesh or an association of a group of particles. So far, this has not been reported in the literature. All of these processes can potentially increase bioaccessibility during digestion, due to increased surface areas enhancing matrix−gastrointestinal secretion contact.

The extent of matrix breakdown apparently depended on the type of products (e.g., fresh papaya, mango, and tomato and their dried products). Fiber in the flesh was fractured in both

Table 2. Moisture Contents of Samples Used in the Study

tomato (%)			mango (%)			papaya (%)		
fresh	juice	dried	fresh	juice	dried	fresh	juice	dried
93.90	94.79	29.26 (111.70)[a]	80.71	94.57	12.57 (128.86)	87.12	94.38	22.13 (130.59)

[a] Hydration capacities of the dried samples are in parentheses.

fresh and dried products. Skins and seeds were less affected than the flesh, which was altered into particles with a wide range of sizes (1−2 cells to large sizes). Matrix separation occurred between fibers during the chewing of fresh mango, yielding thin cell layers attached to a fiber "backbone" (**Figure 2B**), which was not found in the other samples. Finally, none of the fresh fruits produced aggregated chewed particles (**Figures 1**−**3**).

In contrast, the majority of chewed particles from dried samples were re-formed into large particles and appeared as agglomerates (**Figure 4**), regardless of fruit type and participant. Here, the agglomerates were of various particle sizes. Microscopic examinations of the small particles revealed that the actions of teeth were sufficient to fracture fibers inside the dried fruit matrices, similar to that in the fresh samples (left panel, **Figure 4**). It appears that the fractures make the boli from dried samples less sharp during swallowing, because the boli were rolled up as spheres or oval-shaped particles.

The various tooth actions during in vivo chewing lead to simultaneous fracture, puncture, and compression occurring, supporting the important role of tooth cusps in chewing as suggested by Lucas and Luke (*36*). This distinguishes the particle characteristics produced by human chewing from the homogeneous structures obtained from mechanical size reduction methods. The poor nutritional status in the elderly (*55*, *56*) may be partly due to their difficulty in chewing, due to tooth cusp contours failing to serrate food matrices, which could lead to a serious reduction of nutrient bioavailability.

In the present study, the distributions of chewed particle sizes that were found are presented in **Figures 5** and **6**. On the basis of image analyses, the numbers of particles retained in sieves >2.36 mm peaked at sizes of 0.5 and 1 cm for fresh samples, except fresh papaya at 1.5 cm (**Figure 5**). Finer particles retained on the 75−710 μm sieves were <10% w/w (**Figure 6**). Although cell walls of plant material are unaffected by the gastrointestinal enzymes during in vitro digestions (*27*), the key parameter

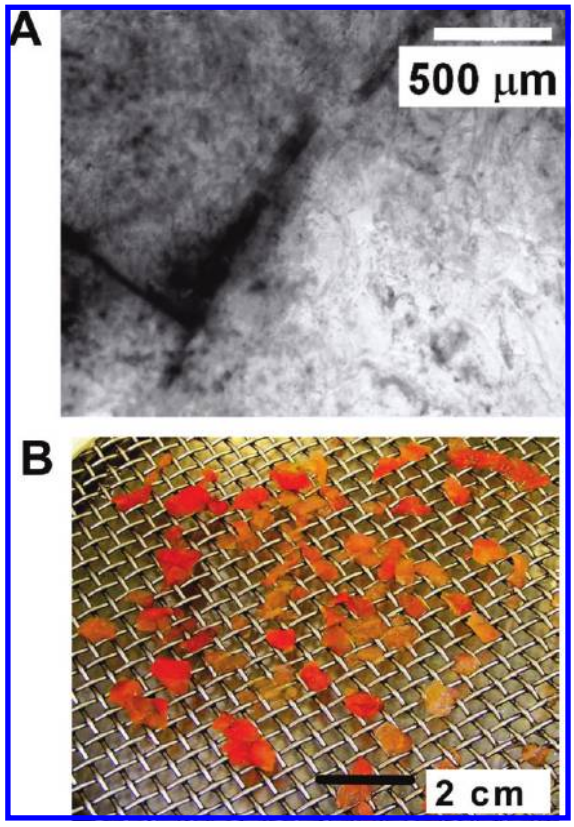


Figure 1. Effects of tooth action during human in vivo chewing of fresh tomato: (**A**) broken fiber in chewed particle observed under a light microscope; (**B**) large chewed particles taken by a camera. The intense red particles were tomato skins

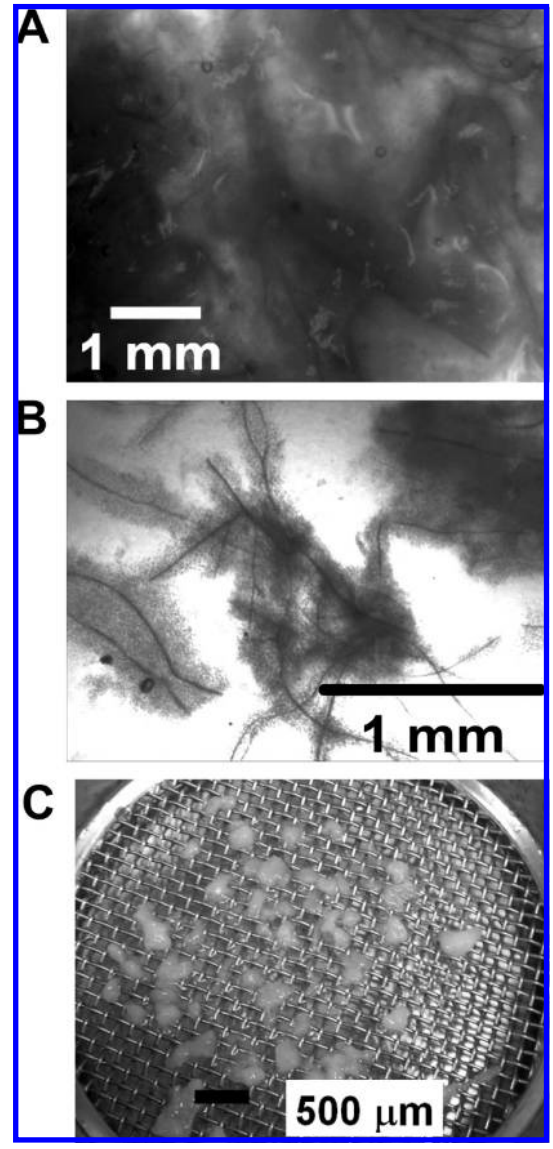


Figure 2. Effects of tooth action during human in vivo chewing of fresh mango: (**A**, **B**) observed under light microscope indicating effects of tooth clenching, fibers were disarranged and exhibited quite a long cut; (**C**) large chewed particles taken by a camera.

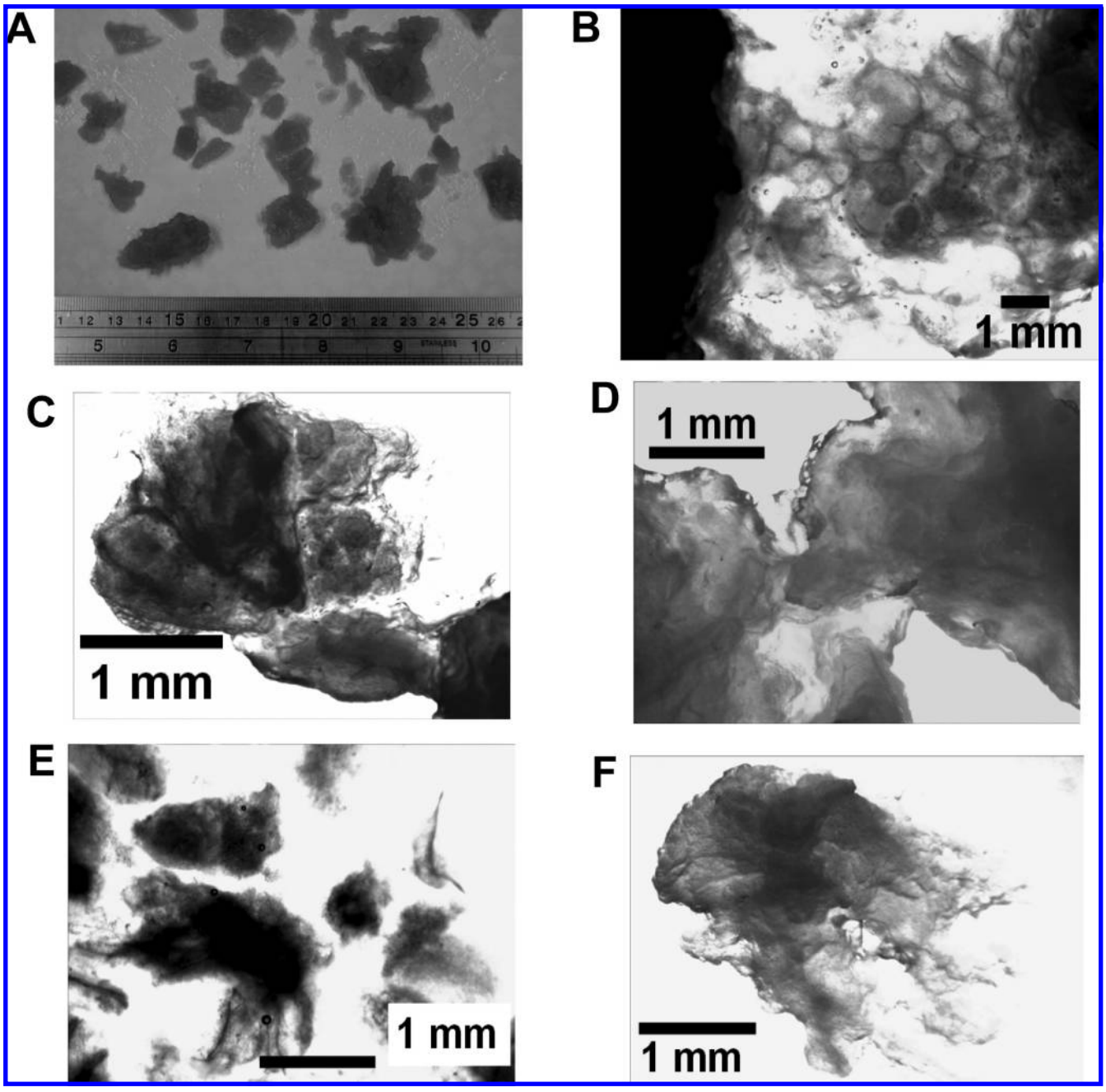


Figure 3. Effects of tooth actions during chewing of fresh papaya: (**A**) large chewed particles (≥1 cm) taken by camera; (**B**−**F**) smaller particles observed under microscope [(**B**) compressed papaya flesh by molar tooth clenching; (**C**−**F**) broken down papaya matrices due to molar tooth contour. Generally, particles were not free particles but incompletely cut from other particles. Water flushing was required to force such particles to avoid mismeasurement when they were retained at a high aperture sieve.

determining bioaccessibility is presumably the degree of intactness of cells (*25*). Therefore, the sample used for in vitro digestion was designed to imitate chewed solid samples as shown under Materials and Methods.

pH Conditions of in Vitro Digestions. pH as an indicator of general conditions of in vitro digestions in the present experiments diverged at the end of each digestion step. Because chewed products are not of a single particle size, mimicked as the simulated particles containing juices and blended and cut particles, the pH diverged possibly because of slow diffusion of nutriome components from the large particles. During in vitro digestion, simulated particles were intact and mostly occupied the bottom of the Falcon tubes as expected, so as to mimic the lower part of the stomach. Although the nutrient-releasing process may be limited by these conditions, such conditions may be realistic (*54*, *55*).

Nutriomic from Tomato. Using the in vitro digestion model developed, the top panel in **Figure 7** shows that digestion releases the major sugars to similar levels from fresh and dried products, but at lower levels than from juice. On the basis of the ratio of extractability for total sugar determination of undigested and digested tomato products, sugars in the digest solutions of tomato products varied from 60% of those extracted from sample control (fresh tomato) to 90% (dried tomato) and 100% (tomato juice; asterisked). The results show a large barrier to sugar release in both fresh and dried products, compared with juice, which can be

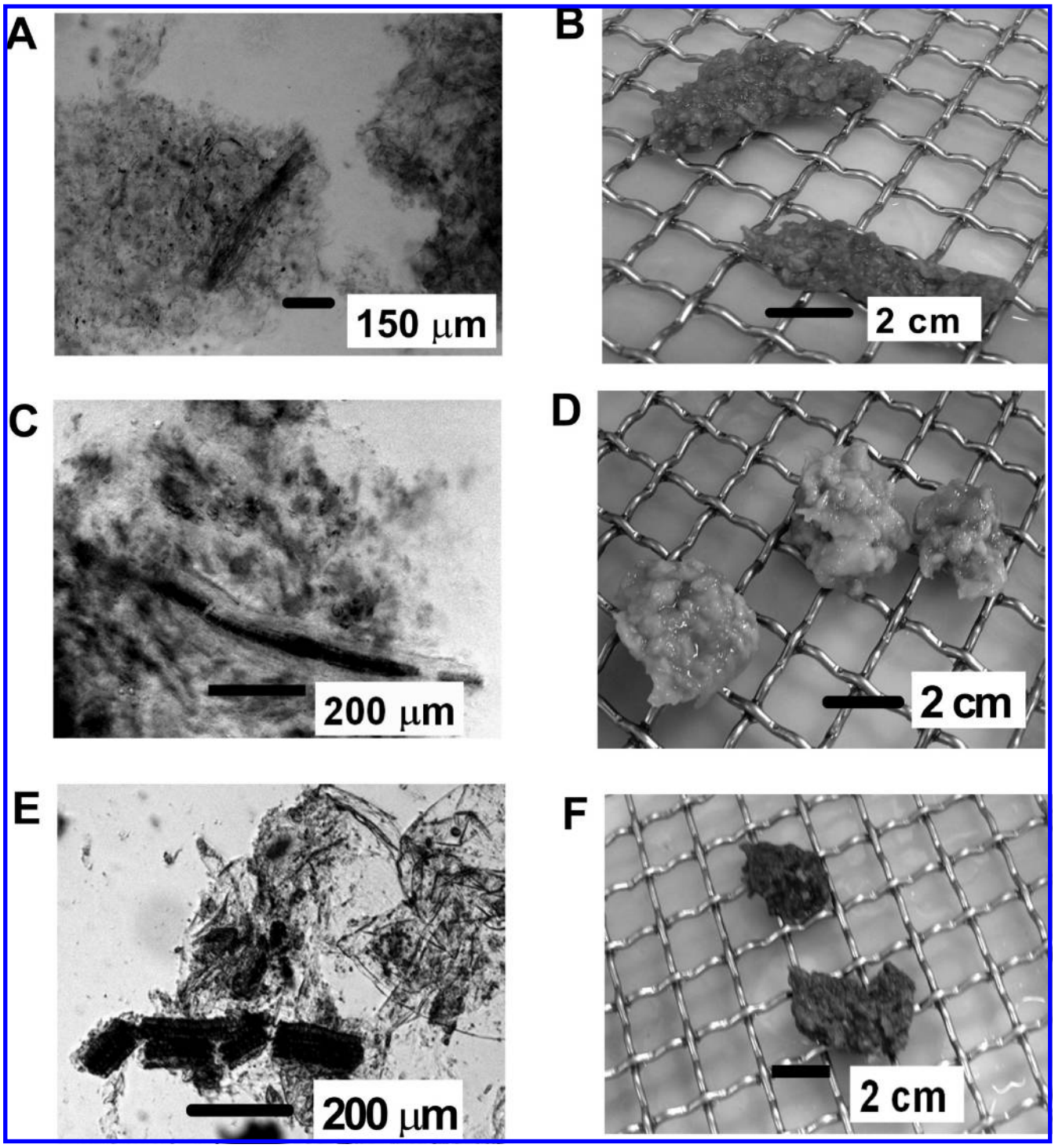


Figure 4. Characteristics of human in vivo chewed materials of dried papaya (**A**, **B**), dried mango (**C**, **D**), and dried tomato (**E**, **F**) without in vitro digestion. Left panels are small particles observed under a light microscope showing cutting effects of tooth clenching on fiber in samples; right panels are large boli formed during in vivo chewing taken by a camera.

considered to be fully released. Dried products had higher released sugar percentages than fresh tomato. This finding has significant consequences for the control of sugar release rates in vivo with implications for glycemic index values and potential long-term effects on diabetes and obesity.

During in vitro digestions (**Figure 7**, middle panel), only 50% of the total extractable carotenoids were released into the digest solutions, which were composed of lycopene and β-carotene in similar proportions. The processing method and the type of digestion, and their interactions, significantly ($p < 0.05$) contributed to carotenoid release. The effects of intestinal digestions (I) on release of carotenoid contents were significantly ($p < 0.05$) higher than that of the gastric digestion (G) and that of the consecutive gastric-intestinal digestion series (G+I). This is because the intestinal digestion (I) had higher concentration gradients due to renewal of the in vitro reagents during each digestion. As a result, the ratio of in vitro bile components to carotenoids was higher compared to those from the other two digestion steps. This likely provides an environment that extracts more carotenoids into the digest solutions, leading to better micelle formation. Micelle formation requires a critical bile−lipid concentration (*59*).

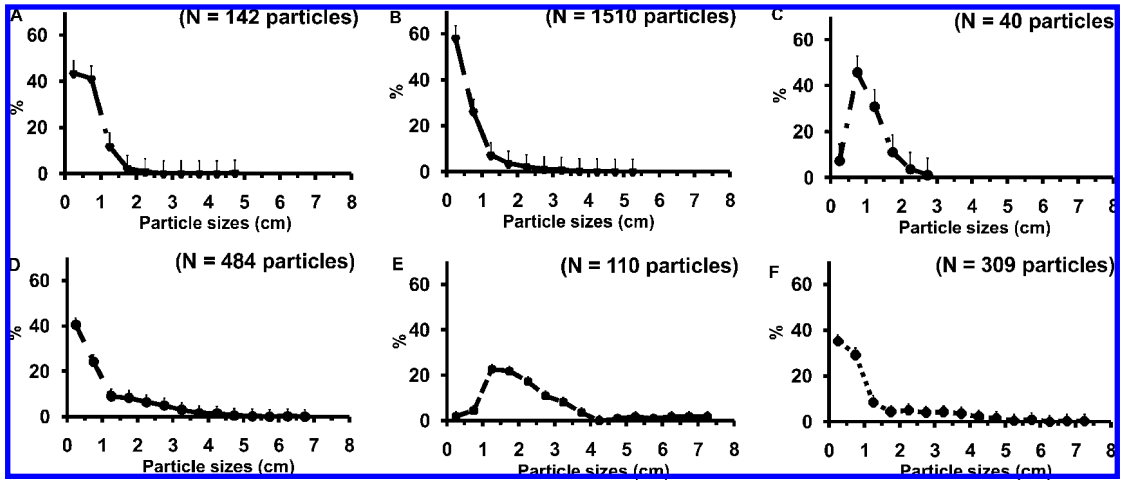


Figure 5. Size distributions of human in vivo chewed samples based on number of particles in each size category: (**A**) fresh tomato; (**B**) dried tomato; (**C**) fresh mango; (**D**) dried mango; (**E**) fresh papaya; (**F**) dried papaya. Data were obtained by using Jimage analysis software.

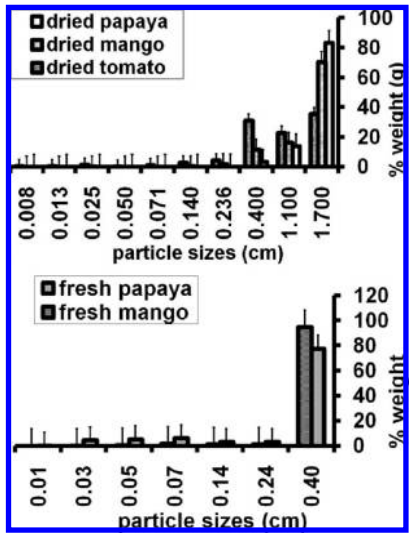


Figure 6. Weight fractions of chewed particles retained at Australian Standard and homemade sieves with apertures of 75−710 μm, 1.4−0.4 mm, and 1.1−1.7 cm for dried (top) and fresh (bottom) samples were calculated from recovered chewed materials during sieving toward spat materials obtained from volunteers. The recoveries were 50−100%. In dried samples, most boli were found to be agglomerated samples (1.1−1.7 cm) and were not completely separated after in vitro digestions producing majority individual particles of 0.4 cm, especially dried papaya boli.

This may have resulted from (a) higher carotenoid concentration gradients between the refreshed reagents and solid particles, in the presence of a bile concentration similar to that of the consecutive gastric-intestine digestion system (G+I), and (b) better contact between the digest reagent and the solid tomato particles because $NaHCO_3$ addition dissolves pectin substances. Because acid prevented pectin solubilization, this may have resulted in limited carotenoid release during gastric digestion. This was observed as agglomerated dried boli went through in vitro digestion steps.

Most organic acids were better released during the consecutive gastric-intestinal digestions (G+I), except for malic acids at gastric digestions (G) were better (**Figure 7**, bottom panel). The level of total acids released during the consecutive gastric-intestinal (G+I) digestions of all products was in the order of juice > dried > fresh. During in vitro digestion there was generally twice the level of organic acids released from juice as compared with from fresh or dried products, illustrating the barrier properties of the pectin-rich cell walls. Compared to undigested samples, the level of total organic acids released during in vitro digestion of juice and dried products reached the maximum level during the consecutive gastric-intestinal (G+I) digestions, whereas for fresh tomato, higher total acids were released during the gastric (G) digestions. The dried products require rehydration in order to be well digested, leading to a delay in the release of organic acids. Therefore, the organic acids were better released during the consecutive gastric-intestinal (G+I) digestions, suggesting that the pectin physical state changed after $NaHCO_3$ additions in the G+I digestions, leading to a freeing of organic acids. Generally, the organic acids from the fresh tomato were poorly released (only about $^1/_{10}$) during in vitro digestion, whereas the organic acids from the tomato juices and the dried tomato were completely released.

Nutriomic from Mango. During in vitro digestions of mango, generally, significant differences were obtained between intestinal digestion alone and the other digestion steps (**Figure 8**, top panel). This suggests that the renewal of reagents may be generating higher concentration gradients between solid particles and solutions leading to the release of higher amounts of sugars. However, the sugars were released at much lower levels by intestinal digestion (I) than by gastric digestion (G) and the consecutive gastric-intestinal digestions (G+I).

In contrast, there was a significant ($p < 0.05$) effect of processing on sugar release. Fructose and glucose were released more from juices due to the lack of cell wall barriers. Furthermore, examination of sucrose indicated that the effects of gastric digestion of juice were similar to those of the gastric digestion of fresh samples. This did not occur in dried products. Meanwhile, sucrose reached a maximum in simultaneous gastric-intestinal digestions (G+I) when pectin was solubilized into digest solutions after $NaHCO_3$ additions. Overall, total sugar releases from mango products during in vitro digestions were similar because of the nature of mango flesh and its matrix characteristics discussed earlier.

The predominant carotenoid, β-carotene, was solubilized best during intestinal digestion (I) for all of the mango products (**Figure 8**, middle panel). Fresh mango released the highest level β-carotene during the intestinal digestions, whereas mango juice released the lowest ($p < 0.05$). Initial free carotenoids due to "chewing" simulation (severely destroyed mango flesh in **Figure 1**) were less available during gastric digestion (G). In addition,

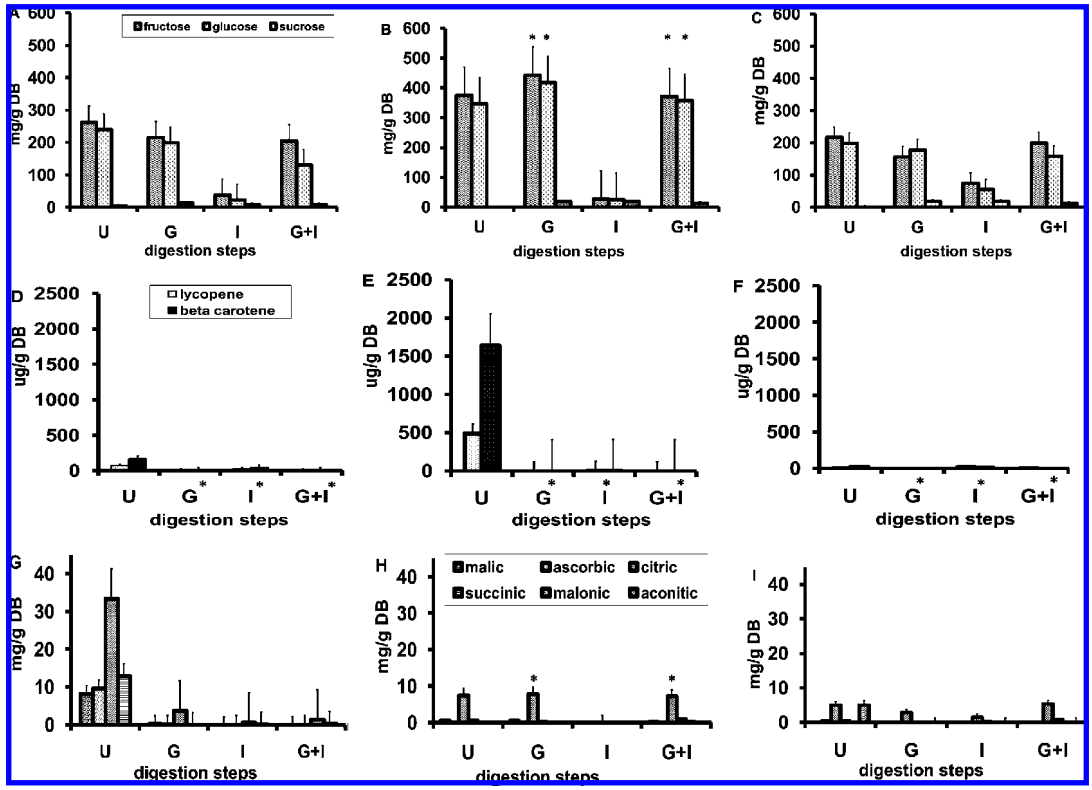


Figure 7. Effects of in vitro digestions on nutriome release from tomato products are significant (asterisked) at $p < 0.05$: sugars (top), major carotenoids (middle), and organic acids (bottom). Sugars, β-carotene, and organic acids are affected significantly by interaction effects of digestion steps and processing. Lycopene is affected significantly by digestion steps. U, undigested; G, gastric digestion; I, intestinal digestion; G+I, consecutive gastric−intestinal digestion. Left column is for fresh tomato, middle is for tomato juice, and right is for dried tomato.

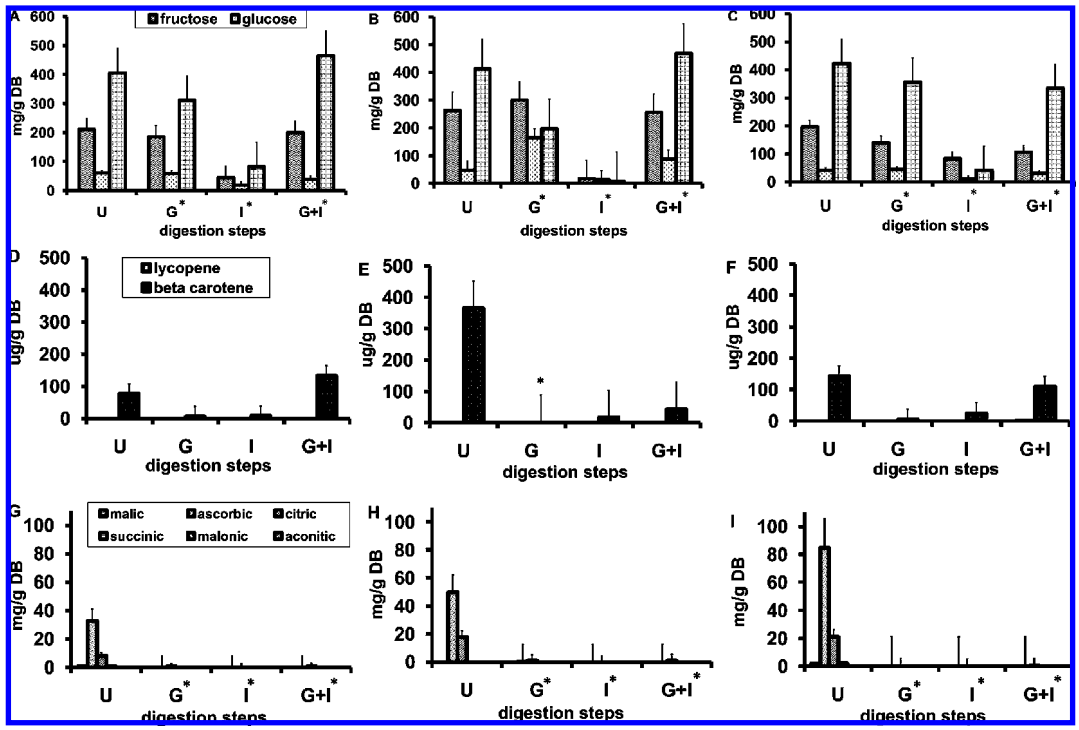


Figure 8. Effects of in vitro digestion on nutriome release from mango products are significant (asterisked) at $p < 0.05$: sugars (top); major carotenoids (middle); organic acids (bottom). Sugars and organic acids are affected significantly by digestion steps, whereas major carotenoids are affected significantly by interaction effects of processing and digestion steps. U, undigested; G, gastric digestion; I, intestinal digestion; G+I, consecutive gastric−intestinal digestion. Left column is for fresh tomato, middle is for tomato juice, and right is for dried tomato.

the mango carotenoids that were not well solubilized, and thus not released, by the consecutive gastric-intestinal digestion (G+I) were released by the intestinal digestion (I). This is probably due to the critical role played by bile components in emulsifying carotenoids. Thus, carotenoids would show a very limited bioavailability from fruit consumption alone.

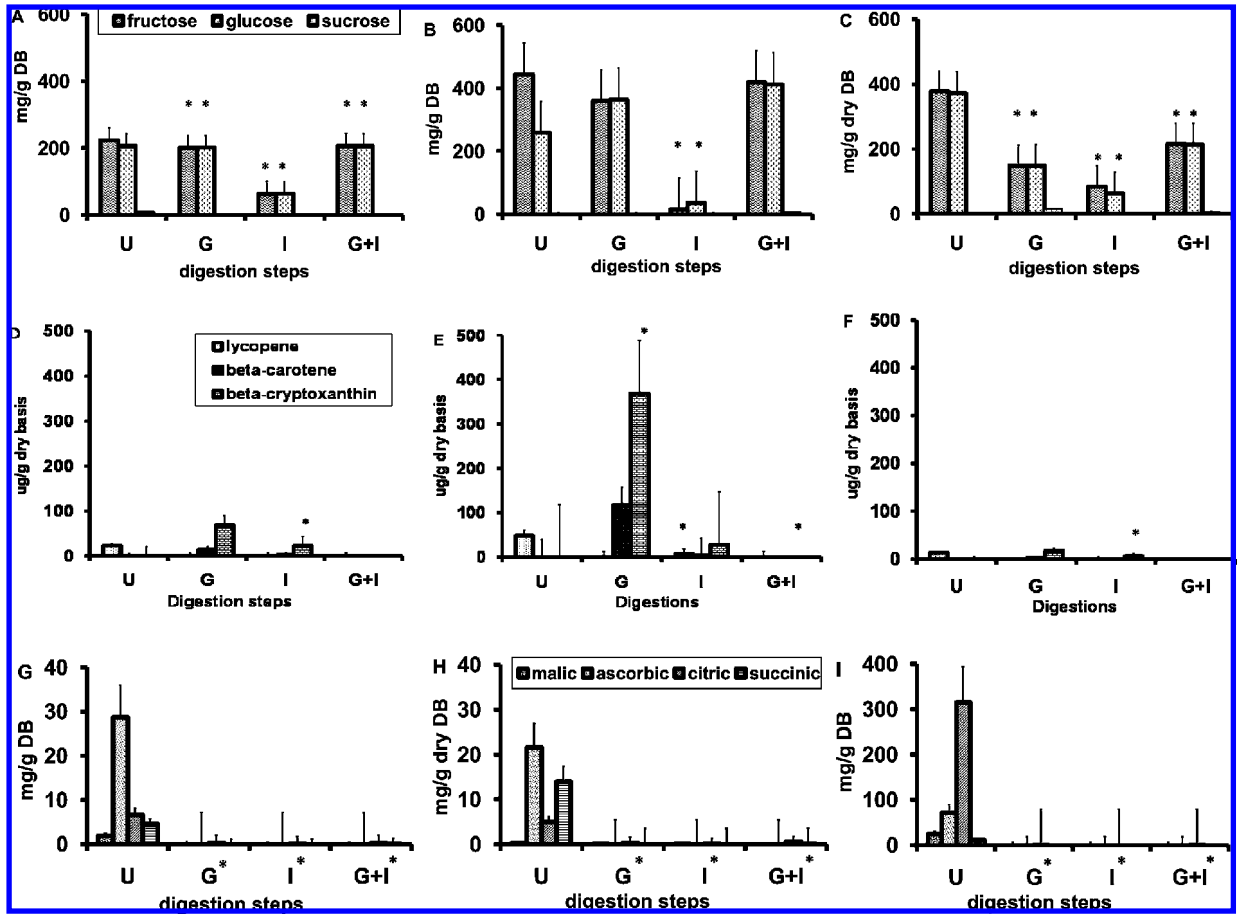


Figure 9. Effects of in vitro digestions on nutriome release from papaya products are significant (asterisked) at $p < 0.05$: sugars (top); major carotenoids (middle); organic acids (bottom). Sugars and carotenoids are affected significantly by interactions of processing and digestion steps. Organic acids are unaffected by all treatments. U, undigested; G, gastric digestion; I, intestinal digestion; G+I, consecutive gastric-intestinal digestion. Left column is for fresh tomato, middle is for tomato juice, and right is for dried tomato.

Drying led to better carotenoid release than juicing, suggesting that a cell wall free food system does not necessarily provide better availability of nonpolar nutriome components. The treatments were significant ($p < 0.05$). During consecutive gastric-intestinal digestions (G+I), fresh and dried mango had comparable levels of released β-carotene. However, the released β-carotene during consecutive gastric-intestinal digestion (G+I) from each product was 18.5% from fresh mango, 7.1% from mango juice and 17.6% from dried mango, respectively. The percentages were compared to the carotenoids found in undigested mango products.

During in vitro digestions, particular organic acids had significantly ($p < 0.05$) different release profiles (**Figure 8**, bottom panel). Generally, the intestinal digestions showed significant ($p < 0.05$) differences in the release profiles for individual organic acids compared to the other digestion steps, which did not. Citric acid and ascorbic acid were affected significantly ($p < 0.05$) by the digestion steps. Citric acid was released readily from gastric digestions (G), and consequently the consecutive gastric-intestinal digestions (G+I) have citric acid levels similar to the gastric digestion (G) (**Figure 9**, bottom panel). The sum of sugars released from gastric (G) and intestinal digestions (I) was not equal to those from consecutive gastric-intestinal digestions; presumably additional release of acids during intestinal digestion (I) resulted from an equilibrium state that was not reached. On the other hand, malic acid was significantly ($p < 0.05$) affected by processing and by the digestion steps. In particular, the level of released malic acid is higher from fresh mango than from dried mango, presumably because the slow rehydration rate of the dried mango delayed diffusion and subsequent extraction during digestion. For ascorbic acid, there was more apparent release from the gastric digestion (G) compared to that from the consecutive gastric and intestinal (G+I) mimic. This was most likely due to the instability of ascorbic acid (due to oxidation) during the longer G+I in vitro digestions. Overall, total acids were affected by digestions and processing, with juice having the highest organic acid release rate.

Nutriomic from Papaya. During in vitro digestions, glucose and fructose were released mainly in gastric digestion (G) or the consecutive gastric-intestinal digestions (G+I) (**Figure 9**, top panel) as expected from their high aqueous solubilities. Sugar release from fresh and dried papaya was consistently about half of that from papaya juice. This suggests that "chewed" papaya released lower levels of sugars than juiced papaya due to the multiple structural barriers of the solid particles and would lead to the prediction of a greater glycemic index for papaya juice compared with either fresh or dried papaya.

In vitro digestions caused only a limited (5−12.5%) release of carotenoids, consistent with literature evidence for the limited bioavailability of these molecules (*60*−*62*). In all cases, release of carotenoids in the gastric digestion was minimal, consistent with the very low aqueous solubility of these compounds (**Figure 9**, middle panel). The emulsifying properties of the bile

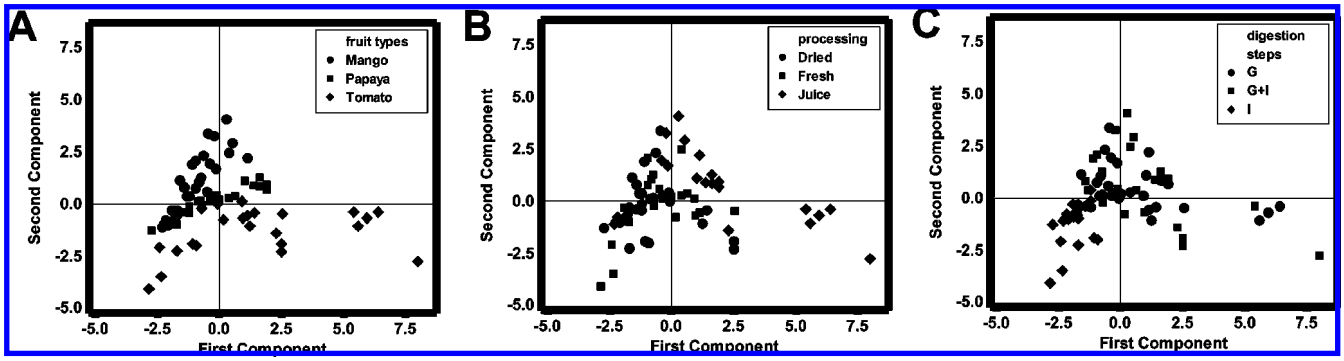


Figure 10. Mapping of nutriome release (all data sets of samples in w/w DB) grouped using PCA (showing 98.70% data set principal components) according to (**A**) fruit types, (**B**) processing, and (**C**) digestion steps. Overall, PCA shows nutrient release from tomato products was highly varied by either processing or digestion steps, whereas that from papaya products was affected least. Intestinal digestion released the least nutrients. For each fruit type, juicing resulted in the highest nutrient release compared to other processing, indicating fruit type is the main factor affecting nutrient release. G, gastric; I, intestine; G+I, consecutive gastric−intestine.

component in intestinal digests appear to be needed for any extraction of carotenoids from papaya.

More carotenoids were released in simulated intestinal digestion (G) than in other digestion steps. β-Carotene and β-cryptoxanthin were not released from papaya juice during gastric and intestinal digestion (G+I). By comparison to lycopene and β-carotene released from tomato, the results suggest that the poor intestinal release of β-carotene and β-cryptoxanthin resulted from a papaya characteristic rather than an intrinsic carotenoid property. Currently, the reason is unknown.

However, total carotenoids showed that intestinal digestion (I) released more carotenoids for all samples. The least available carotenoids were from dried papaya. The predominant β-carotenoids dissolved better in intestinal digestion (I), except those from papaya juice. Fresh papaya released more carotenoids after intestinal digestion (I), whereas papaya juice was the lowest β-carotene. The differences between treatments were significant ($p < 0.05$). For fresh and dried papaya, initial free carotenoids available due to "chewing" simulation were not released during gastric digestion (G), resulting in a low carotenoid availability; conversely, those released during intestinal digestion (I) resulted in higher carotenoid availability. The simultaneous gastric-intestinal digestions (G+I) decreased the available β-carotenoids. Hence, the moderate carotenoid release from the simultaneous gastric-intestinal digestion (G+I) was more likely obtained from intestinal (I) digestion steps. Nevertheless, fresh papaya released higher amounts of β-carotenoids than did dried papaya; endogenous enzymes in fresh papaya may help to destroy the fresh papaya matrix. On the other hand, the effect of in vitro intestinal digestion of papaya juices, which resulted in the lowest amount of β-carotene being released, was against the general trend where the presence of bile increased carotenoid solubility, such as observed for fresh papaya and dried papaya. Possibly, the dissolved pectin, after $NaHCO_3$ addition, bound the bile components, thus making them unavailable for carotenoid-rich micelle formations. Dietary fiber is reported to bind bile, which affects lipid absorption in the small intestine (*63*). Moreover, eating a carotenoid-containing fruit as part of a meal that also contains oils and fats does not always improve micellarization of the released carotenoids, as was found for lycopene from tomato (*64*). In the present research, the released carotenoids from all samples were low (5−12.5%) compared to extractable carotenoid contents in undigested samples. Thus, β-carotenoids are predicted to be supplied to only a limited extent from fruit consumption alone.

Overall, total organic acid releases during in vitro digestion (**Figure 9**, bottom panel) took place primarily under gastric conditions, with similar release levels for fresh and juiced products. Levels released from dried papaya were less than for the other two forms.

Simultaneous Pattern Analysis. The fruits studied in the present research have different matrices and biological structures, but share common nutriome contents. To compare simultaneously all nutriome releases during in vitro digestions from these different food matrices, PCA was used. The analysis shows that two principal components account for 98.70% of data variation, with 65.83% of the variation being associated with the first principal component and 32.87% with the second component. Thus, the graphs in **Figure 10** are representative for the data set analyzed.

When in vitro nutriome release is grouped on the basis of fruit type (**Figure 10A**), individually, tomato, mango, and papaya have specific patterns. This suggests that the release of the nutriome is dependent on fruit type, probably because of the specific chemical compositions of individual nutriomes (e.g., phenolics, organic acids, carotenoid compositions, or different pectin types), and physical structure differences in each fruit. On the basis of the distribution of data coordinates, mango and papaya show limited variation with respect to the parameters studied (composition, digestion step). Conversely, tomato shows a wider range of PCA coordinates, suggesting more sensitivity to the parameters studied in terms of nutriome release.

A plot of the nutriome data sets based on processing, that is, juiced, dried, and unprocessed (fresh) (**Figure 10B**) shows that juicing results in higher nutriome release (more positive coordinate points) than does drying or no processing (fresh). Because the PCA coordinates of the fruit components were extracted from variability data, the origin point can be assumed as the grand mean value of the whole data set. Thus, juicing tends to result in a higher (farther from the origin) nutriome release (measured as w/w DB) than does drying or no processing (fresh), which themselves tended to have similar releases of nutriome.

A further data plot based on in vitro digestion steps (**Figure 10C**) shows that the release of the nutriome for the intestinal digestion (I) differs from those of the gastric digestion (G) and the consecutive gastric-intestinal digestions (G+I), which were themselves similar. Combining information from **Figure 11** shows that nutriome release from tomato juice under gastric digestion (G) or the consecutive gastric and intestinal digestion (G+I) is differentiated from all other types through high values of PCA component 1.

Vector plots for individual nutriome components are shown in **Figure 11**. The vectors indicate the amount of nutriome component release as affected by the latent effects of the

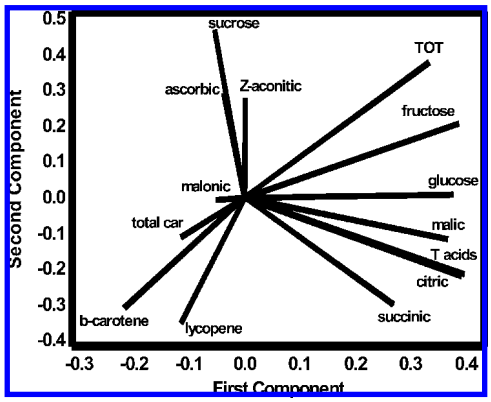


Figure 11. Mapping of nutriome release grouped according to fruit types using PCA (showing 98.70% data set principal components). Sugars and several organic acids were released better than carotenoids, which were mainly released during intestinal digestion. T acid, total acids; total car, total carotenoids.

Table 3. Correlation Table among Individual Nutriomes Studied ($p < 0.05$)

	correlation value		correlation value
glucose−fructose	0.851	total acid−fructose	0.612
sucrose−fructose	0.397	total acid−glucose	0.460
ascorbic−glucose	0.422	total acid−sucrose	0.467
ascorbic−citric	0.498	toatal acid−ascorbic	0.414
citric−fructose	0.586	total acid−citric	0.88
citric−glucose	0.492	total acid−*trans*-aconitic	0.595
citric−sucrose	0.41	β-carotene−fructose	−0.454

parameters studied. Because tomato, mango, and papaya contain similar nutriome compound groups (sugars, organic acids, and carotenoids), it was hypothesized that there would be consistent trends in the experimental results as a function of the processing and digestion steps.

The main understanding obtained from **Figure 11** is that there are three subgroups of nutriome released from the fruits studied in this research: (1) total sugars and organic acid behaved similarly (positive values of component 1) regardless of fruit types; (2) carotenoids as nonpolar compounds increase in concentration during intestinal digestion (I), regardless of the fruit type (negative values of both components 1 and 2); and (3) sucrose, ascorbic acid, and *trans*-aconitic acid behaved similarly (positive values of component 2), presumably because they are from the same fruit type, that is, mango (spots in the vectors belong to mango, **Figure 10**). A negative vector is interpreted as a lower score, and a positive vector means a higher score (*67*). A correlation matrix of the nutriome components studied gave high values mainly for fructose−glucose correlation and for citric acid−total acid correlations (**Table 3**). These are the components detected at high levels in the fruits studied. Citric acid is the main organic acid in the fruits studied; therefore, the total organic acid levels behave similarly to the citric acid levels. Thus, the PCA can extract the main information for the main components in a nutriomic mixture. This method is a promising way to analyze a complex data set obtained in a detailed nutriomic study such as the present one.

Sugars and organic acids were the predominant nutriome components, and parameters studied in this research affected their release levels. Carotenoids (nonpolar nutriome components) gave opposite vectors to sugars and organic acids, meaning the released levels were lower the higher were the release levels of sugars and organic acids. Conversely, increased carotenoid will be obtained when the release of sugars and organic acids is low. However, the data sets for the carotenoids were obtained from intestinal digestion (**I**), whereas the data for sugars and acids were primarily obtained from simulated gastric digestion (**G**). This opposite vector arrangement found by the PCA is therefore a reflection of solubility (sugars and acids are freely soluble, whereas carotenoids are only soluble in the presence of bile emulsifiers).

Contributions of the digestion steps include chewed particles, specific digestive environments, and processing. Controlled particle sizes were subjected to in vitro digestions. From three types of fruits with different biological structures and textures, the effects of in vitro digestions on the release of sugars, carotenoids, and organic acids were different depending on the fruit matrix and type of individual target molecule. Mango structures appeared to be functionally similar in the juice, fresh, and dried forms, implying that soluble pectin (or highly swollen pectin-rich cell walls) is a controlling factor. Tomato showed variability in nutriome bioavailability as a function of process type, whereas papaya products were relatively resistant toward the in vitro digestion steps.

Sugar release during in vitro digestions showed that mango had no significant differences ($p > 0.05$) for process types. This is presumably because fibrous structures were not altered significantly by processing. However, tomato and papaya showed significant differences ($p < 0.05$) in interaction effects between the digestion step and the processing method. Therefore, changes in the method of processing may or may not affect the release process for the nutriome; this will depend on the physical state of the structures of the food matrices and whether this changes the release properties of nutriomes. The release of organic acids was not significantly different ($p > 0.05$) during in vitro digestions for all of the products except for the dominant citric acids.

In conclusion, chewing of fresh and dried Roma tomato, Kent mango, and Red papaya at swallowing point produced various particles dominated by particle sizes of 0.5 cm for dried fruits and 1.5 cm for fresh fruits. The dried fruits formed agglomerated boli after chewing, but they partially disaggregated during in vitro digestions, which indicated roles of pectinic substances depending on types of fruits. These need to be studied further. On the basis of mass fractions, the dominant chewed particles were approximately 90%. Thus, simulation of in vitro digestion is recommended to be 90% particles of either 0.5 or 1.5 cm for dried and fresh fruits, respectively, plus 10% blended particles. It can be concluded that depending on fruit types, cutting or blending alone may not be a realistic chewing mimic for fully ripe fruits containing high pectinic substances. In vitro digestions of such controlled particle sizes from different fruit types resulted in different nutriome release levels. Apparently, cell walls and pectins of mango, papaya, and tomato swelled or dissolved variably in simulated gastric and small intestinal digestive environments, restricting or enhancing nutriome releases. Contributions of cell walls and solubilized pectin need to be investigated further with respect to hydrodynamic, hydrostatic, and osmotic effects from small molecules. Other factors affecting nutriome release during in vitro digestions are interactions of target compounds with polymers (e.g., pectins), solubility of the target compounds, and interactions with gastric and intestinal digest solutions. Nutriomic analysis expressed as PCA maps can be insightful, illustrating all nutriome components released during digestions.

LITERATURE CITED

(1) Doring, F. Nutriomics - komplexe Analyse der Interaktion zwischen Ernahrungsumwelt und biologischer Individualitat. [online] available at http://www.vdoe.de/jt2002-zf-4-doering.htm, 2004.

(2) Mathers, J. C. Chairman's introduction: what can we expect to learn from genomics? *Proc. Nutr. Soc*. **2004**, *63*, 1–4.

(3) Glahn, R. P.; Cheng, Z.; Welch, R. M.; Gregorio, G. B. Comparison of iron biaovailability from 15 rice genotypes: studies using an in vitro digestion/Caco-2 cell culture model. *J. Agric. Food Chem*. **2002**, *50*, 3586–3591.

(4) Liu, C.-S.; Glahn, R. P.; Liu, R. H. Assessment of carotenoid bioavailability of whole foods using a Caco-2 cell cullture model coupled with in vitro digestion. *J. Agric. Food Chem*. **2004**, *52*, 4330–4337.

(5) Oikeh, S.; Menkir, A.; Maziya-Dixon, B.; Welch, R. M.; Glahn, R. P. Assessment of concentrations of iron and zinc and bioavailability iron in grains of early-maturing tropical maize varieties. *J. Agric. Food Chem*. **2003**, *51*, 3688–3694.

(6) Oikeh, S. O.; Menkir, A.; Maziya-Dixon, B.; Welch, R. M.; Glahn, R. P. Assessment of iron bioavailability from twenty elite late-maturing tropical maize varieties using an *in vitro* digestion/Caco-2 cell model. *J. Sci. Food Agric*. **2004**, *84*, 1202–1206.

(7) Wortley, G.; Leusner, S.; Good, C.; Gugger, E.; Glahn, R. Iron availability of a fortified processed wheat cereal: a comparison of fourteen iron forms using an *in vitro* digestion/human colonic adenocarcinoma (Caco-2) cell model. *Br. J. Nutr*. **2005**, *93*, 65–71.

(8) Ferruzzi, M. G.; Failla, M. L.; Schwartz, S. J. Assessment of degradation and intestinal cell uptake of carotenoids and chlorophyll derivatives from spinach puree using an in vitro digestion and Caco-2 human cell model. *J. Agric. Food Chem*. **2001**, *49*, 2082–2089.

(9) Garrett, D. A.; Failla, M. L.; Sarama, R. J. Estimation of carotenoid bioavailability from fresh stir-fried vegetables using an *in vitro* digestion/Caco-2 cell culture model. *J. Nutr. Biochem*. **2000**, *11*, 574–580.

(10) Laurent, C.; Besancon, P.; Auger, C.; Rouanet, J.-M.; Caporiccio, B. Grape seed extract affects proliferation and differentiation of human intestinal Caco-2 cells. *J. Agric. Food Chem*. **2004**, *52*, 3301–3308.

(11) Hoebler, C.; Devaux, M.-F.; Karinthi, A.; Belleville, C.; Barry, J.-L. Particle size of solid food after human mastication and *in vitro* simulation of oral breakdown. *Int. J. Food Sci. Nutr*. **2000**, *51*, 353–367.

(12) Gil-Izquierdo, A.; Gil, M. I.; Ferreres, F.; Tomas-Barberan, F. A. In-vitro availability of flavonoids and other phenolics in orange juice. *J. Agric. Food Chem*. **2001**, *49*, 1035–1041.

(13) Gil-Izquierdo, A.; Zafrilla, P.; Tomas-Barberan, F. A. An *in vitro* method to stimulate phenolic compound release from the food matrix in the gastrointestinal tract. *Eur. Food Res. Technol*. **2002**, *214*, 155–159.

(14) Miller, D.; Schricker, B.; Rasmussen, R.; Van Campen, D. An *in vitro* method for estimation of iron availability from meals. *Am. J. Clin. Nutr*. **1981**, *34*, 2248–2256.

(15) Schricker, B.; Miller, D.; Rasmussen, R.; Van Campen, D. A comparison of *in vivo* and *in vitro* methods for determining availability of iron from meals. *Am. J. Clin. Nutr*. **1981**, *34*, 2257–2263.

(16) Swain, J. H.; Newman, S. M.; Hunt, J. R. Bioavailability of elemental iron powders to rats is less than bakery-grade ferrous sulfate and predicted by iron solubility and particle surface area1. *J. Nutr*. **2003**, *133*, 3546.

(17) Artursson, P.; Palm, K.; Luthman, K. Caco-2 monolayers in experimental and theoretical predictions of drug transport1. *Adv. Drug Delivery Rev*. **2001**, *46*, 27–43.

(18) Bissonnette, P.; Gagne, H.; Coady, M. J.; Benabdallah, K. Kinetic separation and characterization of three sugar transport modes in Caco-2 cells. *Am. J. Physiol*. **1996**, *33*, G833.

(19) During, A.; Albaugh, G.; Smith, J. C. Charactrization of β-carotene 15,15′-dioxygenase activity in TC7 clone of human intestinal cell line Caco-2. *Biochem. Biophys. Res. Commun*. **1998**, *249*, 467–474.

(20) Galijatovic, A.; Otake, Y.; Walle, U. K.; Walle, T. Induction of UDP-glucuronosyltransferase UGT1A1 by the flavonoid chrysin in Caco-2 cells—potential role in carcinogen bioinactivation. *Pharm. Res*. **2001**, *18*, 374–379.

(21) Gan, L. S. L.; Moseley, M. A.; Khosla, B.; Augustijns, P. F.; Bradshaw, T. P.; Hendren, R. W.; Thakker, D. R. CYP3A-like cytochrome P450-mediated metabolism and polarized efflux of cyclosporin a in Caco-2 cells: interaction between the two biochemical barriers to intestinal transport. *Drug Metab. Dispos*. **1996**, *24*, 344–349.

(22) Herrera-Ruiz, D.; Wang, Q.; Cook, T. J.; Knipp, G. T.; Gudmundsson, O. S.; Smith, R. L.; Faria, T. N. Spatial expression patterns of peptide transporters in the human and rat gastrointestinal tracts, Caco-2 *in vitro* cell culture model, and multiple human tissues. *AAPS Pharmsci* **2001**, *3*, E9.

(23) Levin, M.; Davis, A. Retinoic acid increases cellular retinol binding protein II mRNA and retinol uptake in the human intestinal Caco-2 cell line. *J. Nutr*. **1997**, *127*, 13–17.

(24) Parada, J.; Aguilera, M. J. Food Microstructure affects the bioavailability of several nutrients. *J. Food Sci*. **2007**, R21−32.

(25) Tydeman, E.; Wickham, M.; Faulks, R.; Parker, M.; Waldron, K.; Fillery-Travis, A.; Gidley, M. Plant cell walls—a barrier to bioaccessibility of organic micronutrients. In *SIK-Dokument*; Kompediet: Goteborg, Sweden, 2003.

(26) Castenmiller, J. J. M.; West, C. E.; Linssen, J. P. H.; van hef Hof, K. H.; Voragen, A. G. J. The food matrix of spinach is a limiting factor in determining the bioavailability of (β-carotene) and to a lesser extent of lutein in humans. *J. Nutr*. **1999**, *129*, 349.

(27) Miller, J. G.; Buchanan, C. J.; Eastwood, M. A.; Fry, S. C. The solubilisation and hydrolysis of spinach cell wall polysaccharides in gastric and pancreatic fluids. *J. Sci. Food Agric*. **1995**, *68*, 389–394.

(28) Read, N. W.; Welch, I. M.; Austen, C. J.; Barnish, C.; Bartlett, C. E.; Baxter, A. J.; Brown, G.; Compton, M. E.; Hume, K. E.; Storie, I.; Worlding, J. Swallowing food without chewing; a simple way to reduce postprandial glycaemia. *Br. J. Nutr*. **1986**, *55*, 43–47.

(29) Fatema, K.; Ali, L.; Rahman, M. H.; Parvin, S.; Hassan, M. Z. Serum glucose and insulin response to mango and papaya in type 2 diabetic subjects. *Nutr. Res. (N.Y.)* **2003**, *23*, 9–14.

(30) Guevarra, M. T. B.; Panlasigui, L. N. Blood glucose responses of diabetes mellitus type II patients to some local fruits. *Asia Pac. J. Clin. Nutr*. **2000**, *9*, 303–308.

(31) Bosman, F.; van der Bilt, A.; Abbink, J.; Van der Glas, H. W. Neuromuscular control mechanisms in human mastication. *J. Texture Stud*. **2004**, *35*, 201–221.

(32) Lucas, P. W.; Prinz, J. F.; Agrawal, K. R.; Bruce, I. C. Food texture and its effects on ingestion, mastication and swallowing. *J. Texture Stud*. **2004**, *35*, 159–170.

(33) Hiiemae, K. Mechanisms of food reduction, transport and deglutition: how the texture of food affects feeding behaviour. *J. Texture Stud*. **2004**, *35*, 171–200.

(34) Jiffry, M. Analysis of particles produced at the end of mastication in subjects with normal dentition. *J. Oral Rehab*. **1981**, *8*, 113–119.

(35) Jiffry, M. Variations in articles produced at the end of mastication in subjects with different types of dentition. *J. Oral Rehab*. **1983**, *10*, 357–362.

(36) Lucas, P. W.; Luke, D. A. Methods for analysing the breakdown of food in human mastication. *Arch. Oral Biol*. **1983**, *28*, 813–819.

(37) Lucas, P. W.; Luke, D. A. Is food particle size a criterion for the initiation of swallowing? *J. Oral Rehab*. **1986**, *13*, 127–136.

(38) Mioche, L.; Bourdiol, P.; Sandra, M. Chewing behaviour and bolus formation during mastication of meat with different textures. *Arch. Oral Biol*. **2003**, *48*, 193–200.

(39) Gambareli, F. A.; Serra, M. D.; Perreira, L. J.; Gaviao, M. B. D. Influence of measurement technique, test food, teeth and muscle

force interactions in masticatory performance. J. Texture Stud. **2007**, *38*, 2–20.
(40) Etcheverry, P.; Miller, D. D.; Glahn, R. P. A low-molecular-weight factor in human milk whey promotes iron uptake by Caco-2 cells. J. Nutr. **2004**, *134*, 93.
(41) Glahn, R. P.; Wien, E. M.; van Campen, D. R.; Miller, D. D. Caco-2 cell iron uptake from meat and casein digests parallels *in vivo* studies: use of a novel *in vitro* method for rapid estimation of iron bioavailability. J. Nutr. **1996**, *126*, 332–339.
(42) Glahn, R. P.; Lai, C.; Hsu, J.; Thompson, J. F. Decreased citrate improves iron availability from infant formula: application of an *in vitro* digestion/Caco-2 cell culture model. J. Nutr. **1998**, *128*, 257.
(43) Glahn, R. P.; Lee, O. A.; Yeung, A.; Goldman, M. I.; Miller, D. D. Caco-2 cell ferritin formation predicts nonradiolabeled food iron availability in an *in vitro* digestion/Caco-2 cell culture model. J. Nutr. **1998**, *128*, 1555–1561.
(44) Glahn, R. P.; Lee, O. A.; Miller, D. D. *In vitro* digestion/Caco-2 cell culture model to determine optimal ascorbic acid to Fe ratio in rice cereal. J. Food Sci. **1999**, *64*, 925–928.
(45) Glahn, R. P.; Wortley, G. M.; South, P. K.; Miller, D. D. Inhibition of iron uptake by phytic acid, tannic acid, and $ZnCl_2$: studies using an in vitro digestion/Caco-2 cell model. J. Agric. Food Chem. **2002**, *50*, 390–395.
(46) Huh, E. C.; Hotchkiss, A.; Brouillette, J.; Glahn, R. P. Carbohydrate fractions from cooked fish promote iron uptake by Caco-2 cells. J. Nutr. **2004**, *134*, 1681–1689.
(47) Yeung, A. C.; Glahn, R. P.; Miller, D. D. Dephosphorylation of sodium caseinate, enzymatically hydrolyzed casein and casein phosphopeptides by intestinal alkaline phosphatase: implications for iron availability. J. Nutr. Biochem. **2001**, *12*, 292–299.
(48) Yun, S. M.; Habicht, J. P.; Miller, D. D.; Glahn, R. P. An *in vitro* digestion/Caco-2 cell culture system accurately predicts the effects of ascorbic acid and polyphenolic compounds on iron bioavailability in humans. J. Nutr. **2004**, *134*, 2717–2721.
(49) Ben-Amotz, A.; Fishler, R. Analysis of carotenoids with emphasis on 9-*cis*-β-carotene in vegetables and fruits commonly consumed in Israel. Food Chem. **1998**, *62*, 515–520.
(50) Leung, V. W.-H.; Darvell, B. W. Artificial salivas for *in vitro* studies of dental materials. J. Dentistry **1997**, *25*, 475–484.
(51) Baghurst, P.; Beaumont-Smith, N.; Baghurst, K.; Cox, D. The relationship between the consumption of fruits and vegetables and health status. In *Report to Department of Health and Aged Care and Strategic Intergovernmental Nutrition Alliance*; CSIRO Health Sciences and Nutrition, **1999**.
(52) Silverthorn, D. U. *Human Physiology: An Integrated Approach*; Pearson/Benjamin Cummings: San Fransisco, CA, 2004; pp 667.
(53) Morris, C. R.; Scott, J. T.; Chang, H.-M.; Sederoff, R. R.; O'Malley, D.; Kadla, J. F. Metabolic profiling: a new tool in the study of wood formation. J. Agric. Food Chem. **2004**, *52*, 1427–1434.
(54) Buschang, P. H.; Throckmorton, G. S.; Travers, K. H.; Johnson, G. The effects of bolus size and chewing rate on masticatory performance with artificial test foods. J. Oral Rehab. **1997**, *24*, 522–526.
(55) Agerberg, G.; Carlsson, G. Chewing ability in relation to dental and general health. *Acta Odntol. Scand.* **1981**, *39*, 147–153.
(56) Chen, M.; Lowenstein, F. Masticatory handicap, socieconomic status, and chronic condition among adults. J. Am. Dent. Assoc. **1984**, *109*, 916–918.
(57) Johnson, R. L. *Gastrointestinal Physiology*; Mosby: St. Louis, MO, 2001; pp 1−200.
(58) Kutchai, H. C. *The Gastrointestinal System in Physiology*; Berne, R. M., Ed.; Mosby: St. Louis, MO, 2004; pp 539−620.
(59) Wickham, M.; Garrood, M.; Leney, J.; Wilson, P. D.; Fillery-Travis, A. Modification of phospholipid stabilized emulsion interface by bile salt: effect on pancreatic lipase activity. J. Lipid Res. **1998**, *39*, 623–632.
(60) Bohm, V.; Bitsch, R. Intestinal absorption of lycopene from different matrices and interactions to other carotenoids, the lipid status, and the antioxidant capacity of human plasma. Eur. J. Nutr. **1999**, *38*, 118–125.
(61) Bugianesi, R.; Salucci, M.; Leonardi, C.; Ferracane, R.; Catasta, G.; Azzini, E.; Maiani, G. Effect of domestic cooking on human bioavailability of naringenin, chlorogenic acid, lycopene and β-carotene in cherry tomatoes. Eur. J. Nutr. [NLM - MEDLINE]. **2004**, *43*, 360.
(62) Rock, C. L.; Lovalvo, J. L.; Emenhiser, C.; Ruffin, M. T.; Flatt, S. W.; Schwartz, S. J. Bioavailability of β-carotene is lower in raw than in processed carrots and spinach in women. J. Nutr. **1998**, *128*, 913.
(63) Schneeman, B. O.; Gallaher, D. Effects of dietry fibre on digestive enzyme activity and bile acids in the small intestine. Proc. Soc. Exp. Biol. Med. **1985**, *180*, 409–414.
(64) Roodenburg, A. J. C.; Leenen, R.; Weststrate, J. A.; Tijburg, L. B. M.; van het Hof, K. H. Amount of fat in the diet affects bioavailability of lutein esters but not of α-carotene, β-carotene, and vitamin E in humans. Am. J. Clin. Nutr. **2000**, *71*, 1187–1193.
(65) Tyssandier, V.; Reboul, E.; Dumas, J.-F.; Bouteloup-Demange, C.; Armand, M.; Marcand, J.; Sallas, M.; Borel, P. Processing of vegetable-borne carotenoids in the human stomach and duodenum. Am. J. Physiol. Gastrointest. Liver Physiol. **2003**, *284*, G913–923.
(66) van het Hof, K. H.; Tijburg, L. B. M.; Pietrzik, K.; Weststrate, J. A. Influence of feeding different vegetables on plasma levels of carotenoids, folate and vitamin C. Effect of distruption of vegetables matrix. Br. J. Nutr. **1999**, *82*, 203–212.
(67) Kroonenberg, P. M. Introduction to Biplots for GxE Tables. Center for Statistics. Research Report 51. The University of Queensland, Brisbane, 1997; pp 1−22.

Received for review July 21, 2008. Accepted February 16, 2009.

JF900368P