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In Vitro Study of the Release Properties of Soy−Zein Protein Microspheres with a Dynamic Artificial Digestive System

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The purpose of this work was to study the performance of microspheres of soy protein isolate (SPI), zein, or SPI−zein complex as vehicles of nutraceutical delivery under fasting and prandial conditions in an artificial digestive system (TIM-1). Riboflavin availability for absorption from the small intestine compartments reached 90% of the total load within 4 h, most of it (65−80%) turning up in the jejunum dialysis fluid, suggesting that this segment is the main site of absorption, regardless of the nature of the microspheres. However, the riboflavin concentrations and the availability for absorption profiles depended on microsphere formulation. Release from pure SPI and zein microspheres in the stomach compartment occurred within 15 min. The availability for absorption from both the jejunum and ileum compartment followed first-order kinetics, indicating that the limiting step in nutrient uptake with these two formulations is absorption by passive diffusion. SPI−zein complex microspheres provided sustained release of riboflavin over 4 h and a near-zero-order nutrient availability for absorption profile in both fasting and prandial states. Suspending SPI−zein complex microspheres in yogurt significantly delayed nutrient release, which would increase the likelihood of gastric-sensitive nutrients passing intact into the intestine for absorption. SPI−zein complex microspheres thus show potential for use as nutraceutical delivery vehicles in the creation of novel functional foods.

KEYWORDS: Soy protein; zein; microspheres; artificial digestive system nutraceutical delivery; availability for absorption

INTRODUCTION

In addition to being vital macronutrients, food proteins are ideal encapsulating materials for nutraceutical compounds (e.g., vitamins, minerals, probiotics, and bioactive peptides), owing to their excellent emulsifying and gelling properties, which enable them to form hydrogels or emulsified hydrogels of controlled particle size. Protein hydrogels can stabilize active payload compounds and modulate their release kinetics to avoid overdoses and to maximize health benefits (*1*−*4*). Food protein microcapsules can be added to a variety of food products without interfering with sensory properties and are degradable by digestive enzymes in the human gastrointestinal (GI) tract. Proteins used as microcapsule wall materials are mainly of animal origin and include caseinate, whey protein, and gelatin (*5*−*16*). Plant proteins have received increasing attention in recent years because of their safety (no risk of bovine spongiform encephalopathy), abundance, and relatively low cost. Development of plant-protein-based microcapsules may provide opportunities to offer novel functional foods to consumers, in particular for the vegan diet. Soy protein and zein are two major commercially available plant proteins. However, research on their use as microencapsulating materials is still limited to a few publications (*17*, *18*). Efforts to develop their applications for nutraceutical delivery are even scarcer.

In our previous work, soy protein isolate (SPI), zein, and SPI−zein complex microspheres (15−25 μm) were prepared using a cold gelation method (*19*). Their payload release properties were studied with a dissolution apparatus in simulated gastric and intestinal buffers without digestive enzymes. Riboflavin (vitamin B_2), a water-soluble micronutrient that plays a key role in energy metabolism, was used as a model nutrient. Release of the riboflavin from SPI microspheres was immediate, whereas release from pure zein microspheres was very slow. Kinetic analysis revealed that diffusion was the major mechanism regulating the release of riboflavin from these two protein microspheres. Blending SPI and zein thus provides a convenient method of controlling the diffusion coefficient, swelling behavior, and nutrient release profile of food-protein-based microspheres in simulated GI buffer. SPI−zein complex microspheres with the SPI/zein ratio of 1:1 showed near-zero-order release kinetics in simulated intestinal buffer, which is a desirable characteristic for nutraceutical delivery to

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create novel functional foods that can have physiological benefits and/or reduce the risk of chronic disease.

However, dissolution systems are unable to provide understanding of risks such as dose dumping and the effects of food on bioavailability under specific GI conditions. Moreover, these systems are not truly representative of the continuously changing conditions encountered during passage through the stomach and the gut, and they give no information about nutrient absorption (*20*). An artificial digestive system that simulates the human GI tract with greater accuracy should therefore be used to study the release properties of these SPI- and zein-based microspheres. TIM-1 (*21*, *22*), a computer-controlled multicompartmental dynamic artificial digestive system, allows the best possible simulation of in vivo dynamic physiological processes that occur within the lumen of the stomach and small intestine of humans (*20*, *23*). It does this by reproducing the following conditions: (i) sequential use of enzymes in physiological amounts, (ii) appropriate pH for the enzymes and addition of relevant cofactors such as bile salts and coenzymes, (iii) removal of the products of digestion, (iv) appropriate mixing at each stage of digestion, (v) controlled physiological transit times for each step of digestion, and (vi) peristaltic action. These requirements are essential for simulating in vivo conditions with an in vitro digestive system. In addition, this in vitro system offers advantages over in vivo studies, such as accuracy, reproducibility (no biological variation), easy manipulation, the possibility of collecting samples at any level of the GI tract at any time during digestion, and freedom from ethical constraints, even when toxic compounds are involved (*20*). Experiments show that the conditions simulated in the TIM-1 are reliably reproduced and consistent with in vivo data (*22*, *24*). Validation experiments demonstrate the predictive value of the system with regard to the availability of minerals (*25*), vitamins (*26*), and food mutagens (*27*) for absorption.

The purpose of the present work is therefore to further our study of the feasibility of using microspheres made of SPI and zein as nutraceutical delivery vehicles, by observing the kinetics of payload release under fasting and prandial conditions of product administration, that is, with water and with a semiliquid food (yogurt as an example) in the presence of digestive enzymes in the TIM-1 artificial digestive system.

MATERIALS AND METHODS

SPI containing 94.4% protein (dry mass) based on macro-Kjeldahl nitrogen determination (AOAC, 1984) using an N factor 6.25 was obtained from Protient Inc. (St. Paul, MN). Soybean oil used to form the emulsions was purchased locally (Merit selection, Canada). Calcium carbonate (40 nm) was kindly provided by NanoMaterials Technology Pte Ltd. (Singapore). Zein protein, pepsin 1:60000 (from porcine stomach mucosa, crystallized and lyophilized), Span 80, Tween 80, and riboflavin (purity ≥ 98%) were purchased from Sigma Chemical Co. (St. Louis, MO). Pancreatin 5× (from hog pancreas) was purchased from ICN Nutritional Biochemicals (Cleveland, OH). All other chemicals were of reagent grade.

Microsphere Preparation and Characterization. SPI microspheres, zein microspheres, and SPI−zein complex (1:1) microspheres were prepared using the method described in our previous work (*19*). After preparation, the microspheres were freeze-dried and stored at 4 °C in plastic bags before use. Their size range (freshly prepared samples) is 15−25 μm based on static light scattering using a Mastersizer 2000 (Malvern Instruments, Southborough, MA). Scanning electron micrographs showed that SPI microspheres and SPI−zein complex microspheres were smooth-surfaced and spherical, whereas zein microspheres displayed a rough surface (*19*). The loading efficiency of the riboflavin (selected nutrient model) obtained with this encapsulation system was about 9.5%.

Table 1. Fasting and Prandial State Parameters for Digestive Simulations Using TIM-1

condition	fasting state experiment	prandial state experiment
gastric compartment		
time (min)/pH	0/4.5	0/6
	10/3.2	15/5.7
	20/2.4	45/4.5
	40/1.8	90/2.9
	60/1.6	120/2.3
	90/1.5	240/1.8
	240/1.5	
secretions	0.25 mL/min pepsin	0.25 mL/min pepsin
	0.25 mL/min lipase	0.25 mL/min lipase
emptying halftime	20 min	70 min
duodenal compartment		
pH	maintained at 6.4	maintained at 6.4
secretions	0.5 mL/min bile solution	0.5 mL/min bile solution
	0.25 mL/min pancreatin solution	0.25 mL/min pancreatin solution
	0.25 mL/min intestinal electrolyte solution	0.25 mL/min intestinal electrolyte solution
jejunal compartment		
pH	maintained at 6.9	maintained at 6.9
dialysis	10 mL/min jejunal fluid solution	10 mL/min jejunal fluid solution
ileal compartment		
pH	maintained at 7.2	maintained at 7.2
dialysis	10 mL/min ileal fluid solution	10 mL/min ileal fluid solution

Artificial Digestive System (ADS). A computer-controlled multicompartmental dynamic system (TIM-1) that simulates human GI tract conditions was used. This system, developed at TNO Nutrition and Food Research (Zeist, The Netherlands), was validated and optimized in collaboration with the Université Clermont1 ERT CIDAM group. TIM-1 includes four compartments in series simulating the stomach, the duodenum, the jejunum, and the ileum. All system parameters (summarized in **Table 1**) were adjusted to simulate typical GI tract conditions in the fasting and prandial states in healthy adults (*28*−*31*). The availability of riboflavin for absorption was estimated from its concentration in the dialysis fluids, into which it entered by passive diffusion from the jejunum and ileum compartment liquids through a hollow fiber membrane (HG 600, HOSPAL COBE, France).

To study the availability for absorption in the fasting state, riboflavin (~400 mg) encapsulated in protein microspheres was introduced into the gastric compartment simultaneously with 200 g of water. One milliliter of liquid was collected from each compartment at time intervals suitable for monitoring typical GI tract physiological processes. Gastric compartment liquid was thus sampled at 10, 20, 30, and 45 min, whereas the duodenal compartment was sampled at 10, 20, 30, 45, 60, 90, and 120 min. The jejunum and ileum compartment contents were sampled at 30, 45, 60, 90, 120, 180, and 240 min. Liquid discharged from the ileum compartment was sampled at 60, 120, 180, and 240 min. The jejunum and ileum dialysis fluids were sampled at 30, 60, 90, 120, 180, 210, and 240 min with careful measurement of each sample volume and the total dialysis fluid volume. Pure riboflavin powder dissolved in saline was also run through the system as a control. The riboflavin availability for absorption was expressed as cumulative amounts of riboflavin in jejunum or ileum dialysis fluid. Total availability for absorption was expressed as the cumulative combined amounts in the jejunum and ileum dialysis fluids.

To study riboflavin availability for absorption from SPI−zein complex microspheres under prandial conditions, the protein microspheres were introduced into the gastric compartment simultaneously with 150 g of yogurt and 150 g of saline solution. All sampling and calculations were done as described for the fasting condition. The riboflavin concentration in the simulated compartments was not evaluated under prandial conditions because the viscosity of the yogurt made the sampling difficult.

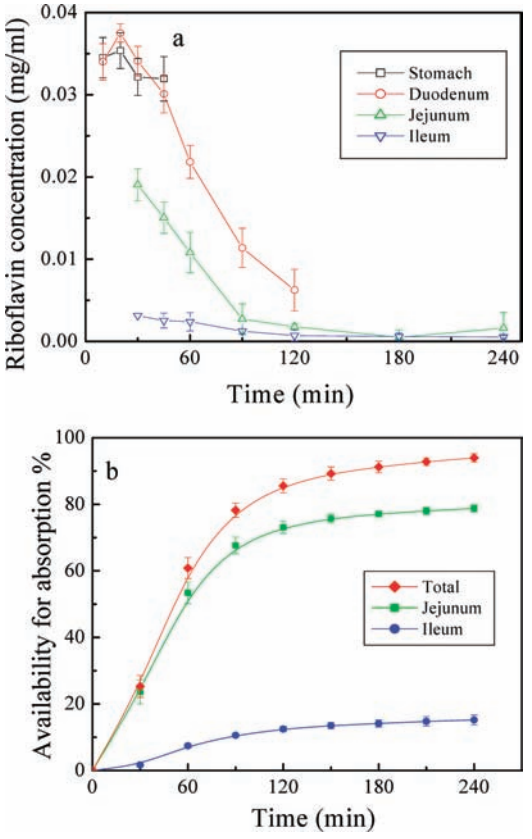

Figure 1. Riboflavin concentration (**a**) and availability for absorption profiles (**b**) in the TIM-1 gastrointestinal tract compartments after feeding free riboflavin dissolved in saline. The values are means ± SD (n = 3).

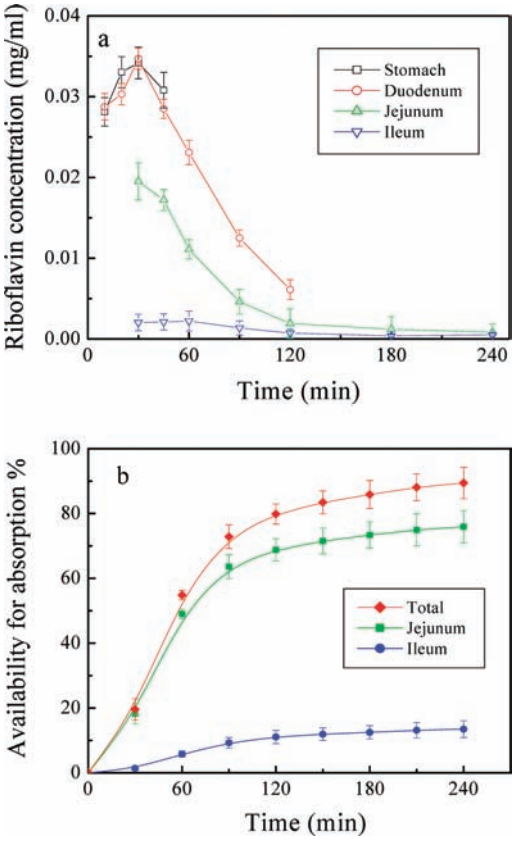

Figure 2. Riboflavin concentration (**a**) and availability for absorption profiles (**b**) in the TIM-1 gastrointestinal tract compartments after feeding riboflavin-loaded SPI microspheres. The values are means ± SD (n = 3).

The recovery (mass balance) of riboflavin was calculated by using the formula

$$\text{recovery}\ (\%) = (\text{riboflavin}_{\text{dialysate}} + \text{riboflavin}_{\text{ileal discharge}} + \text{riboflavin}_{\text{residues}})/\text{riboflavin}_{\text{microsphere}} \times 100$$

where $\text{riboflavin}_{\text{dialysate}}$ is the riboflavin content in jejunal plus ileal dialysis tube, $\text{riboflavin}_{\text{ileal discharge}}$ is the riboflavin content in the total material collected behind the ileocecal valve, $\text{riboflavin}_{\text{residues}}$ is the riboflavin content in the residues collected from the TIM system after ending the experiment, and $\text{riboflavin}_{\text{microsphere}}$ is the riboflavin content in the test protein microspheres.

Riboflavin Analysis. Riboflavin concentration in the gastric and intestinal compartments and the dialysis fluids was analyzed using a Merck-Hitachi LaChrom Elite HPLC (Merck, Darmstadt, Germany) consisting of a pump (model L-2130), an autosampler (model L-2200), and a diode array UV−visible detector (model L-2450). After centrifugation at 10000g and filtration through a 0.45 μm membrane, the collected samples were injected into a C-18 column (Bondapak, 3.9 × 300 mm) at room temperature. The elution solvents used were 0.5 M KH_2PO_4, pH 7.0 (solvent A), and 100% methanol (solvent B) (*32*). The samples were eluted using the following gradient: 1% B for 5 min, 1−30% B (linear gradient) over 15 min, then 30% B for 5 min. The flow rate was 1 mL/min. Riboflavin was detected at 267 nm, and its concentration was calculated from the integrated areas of the sample. This method is sensitive and specific for riboflavin, and as low as 10 ng of riboflavin could be detected reliably (*33*). The blank riboflavin dissolved in KH_2PO_4 at concentrations of (1.2×10^{-4}−4.6×10^{-2} g/L) was also injected in the HPLC system to construct calibration curves.

Statistical Analysis. Three independent TIM runs were conducted on different days for each experiment. Riboflavin samples collected in each run were injected in the HPLC system for concentration determination in duplicates. Data points are the mean of three independent runs ± SD. Statistical significance of the differences was determined by Student's t test. The level of significance used was $p < 0.05$.

RESULTS AND DISCUSSION

Fasting State Experiments. The recoveries for free riboflavin and SPI, zein, and SPI−zein complex microspheres were 104 ± 2, 102 ± 4, 94 ± 5, and 96 ± 6%, respectively, in the fasting state. The recovery of riboflavin for SPI−zein complex microspheres under prandial conditions was 98 ± 1%. The recoveries were relatively complete. For all samples (**Figure 1**−**4**), total riboflavin availability for absorption reached 90% after 4 h, whereas < 10% was in the discharge from the ileum compartment, indicating that riboflavin could be almost completely absorbed in the simulated small intestine. The majority (65−80%) of the riboflavin was recovered in the jejunum dialysis fluid, suggesting that this portion of the small intestine is the main site of absorption, regardless of the nature of the microspheres. However, riboflavin concentration in and the availability for absorption from the four GI tract segments (stomach, duodenum, jejunum, and ileum) during digestion did depend on microsphere formulation. Nutrient absorption in vivo depends on two important factors, namely, solubility and intestinal permeability (*34*). Riboflavin is a low molecular weight water-soluble nutrient and readily permeates the intestine. The solubility of riboflavin is relatively low (~0.1 mg/mL), but the experiment was conducted under strict sink conditions, thus avoiding problems associated with solubility and intestinal permeability. Given the large volume of dialysis fluid used, riboflavin liberated in the jejunum and ileum sections of the TIM-1 should be absorbed rapidly. The concentration and availability for absorption profiles therefore accurately reflect the riboflavin release properties of the protein microspheres in these simulated GI tract sections.

The concentration of free riboflavin dissolved in saline solution passing though the TIM-1 apparatus during the digestion

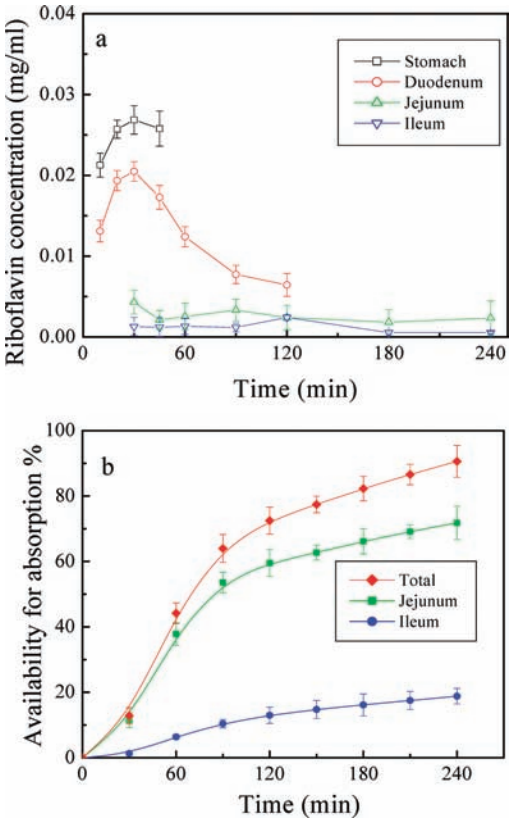

Figure 3. Riboflavin concentration (**a**) and availability for absorption profiles (**b**) in the TIM-1 gastrointestinal tract compartments after feeding riboflavin-loaded zein microspheres. The values are means ± SD ($n = 3$).

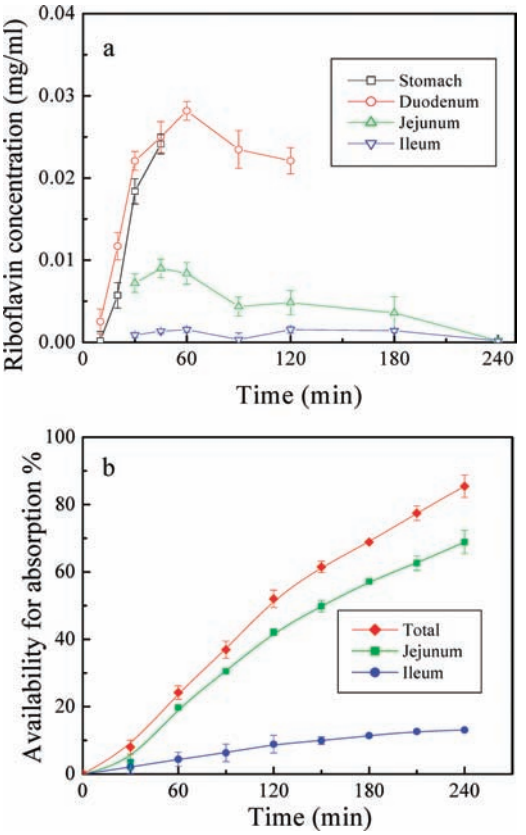

Figure 4. Riboflavin concentration (**a**) and availability for absorption profiles (**b**) in the TIM-1 gastrointestinal tract compartments after feeding riboflavin-loaded SPI−zein complex microspheres. The values are means ± SD ($n = 3$).

program corresponding to fasting conditions is shown in **Figure 1a**, whereas **Figure 1b** shows the availability for absorption of the riboflavin into the dialysis fluids. **Figure 2** shows the same measurements for riboflavin encapsulated in SPI microspheres. In this case, the riboflavin concentrations measured in the gastric and duodenal compartments followed a similar profile, both reaching 2.8×10^{-2} mg/mL after 10 min of digestion and around 3.5×10^{-2} mg/mL within 20−30 min. A significant decrease of the riboflavin concentration in the duodenal compartment was observed only after 45 min of digestion ($p < 0.05$). Riboflavin concentration in the jejunum compartment was low by comparison ($p < 0.05$), reaching 2.0×10^{-2} mg/mL at 30 min and then gradually decreasing. Riboflavin concentrations were the lowest in the ileum compartment ($p < 0.05$), where they stayed below 3×10^{-3} mg/mL for the entire test period. The jejunum and ileum dialysis fluids already contained 77% of the riboflavin after only 1.5 h. This value increased to just 89.4% in the following 2.5 h. By this time, 75.9 and 13.5% were absorbed in the jejunum and ileum compartments, respectively; 3.2% had been discharged from the ileum compartment, whereas 7.4% remained in the small intestinal liquids. The profiles of the availability for absorption featured an initial rapid phase followed by a leveling off. The increased rate of the availability for absorption from the jejunum and the ileum compartments can be modeled according to first-order kinetics ($r^2 \geq 0.998$) in both cases. It is noted that SPI microspheres and free riboflavin gave quite similar distributions of riboflavin concentration in the simulated GI tract and the availability for absorption profiles. This suggests that riboflavin release from SPI microspheres would occur very quickly after oral administration, and the majority of the riboflavin was released in the gastric compartment. Upon reaching the jejunum compartment, the riboflavin could be absorbed, and its concentration in these three compartments decreased gradually. Because most of it was supposed to be absorbed in jejunum, very little reached the ileum compartment, resulting in low concentrations there. The first-order kinetics of the availability for absorption in both the jejunum and ileum compartments indicate that the limiting step in nutrient uptake from SPI microspheres is absorption by passive diffusion (Fick's law) and not release from the microsphere (*20*), further corroborating the immediate release of riboflavin from the SPI matrix. Results obtained using the TIM-1 system are in agreement with our previous findings using a dissolution apparatus, in which a two-phase pattern of riboflavin release was observed for SPI microspheres, consisting of a rapid burst release (~50%) within 15 min in both simulated gastric and intestinal media, followed by slow riboflavin release during the subsequent hours. Diffusion and matrix degradation are two important mechanisms contributing to nutrient release from food protein matrices (*35*). In dissolution experiments without digestive enzymes, initial riboflavin release was limited by time required for dry SPI microspheres to swell in the medium. The hydrophilic SPI matrix changed to a gel state upon hydration, increasing the mobility of the protein molecular chains and allowing the active agent to diffuse outward. The calculated diffusion coefficients for pure SPI microspheres were relatively high (4.7×10^{-8} and 6.8×10^{-8} cm^2/s in pH 1.2 and 7.4 buffers, respectively) (*19*). In the presence of the digestive enzymes (pepsin, trypsin, and pancreatin) in the TIM-1 experiment, protein matrix degradation no doubt contributed to core release in addition to nutrient diffusion, thus leading to very rapid riboflavin release at the initial stage of the test. Our dissolution apparatus work and TIM-1 experiments both support the conclusion that soy protein has relatively poor barrier properties against burst release of encapsulated hydrophilic

nutrients from matrices in the micrometer size range. Although riboflavin is stable in the gastric compartment and would reach the artificial small intestine for absorption, many payloads cannot survive gastric pH and pepsin attack, for example, probiotics and bioactive peptides. The too rapid core release of ingredient release from the microspheres in the stomach compartment therefore makes SPI unsuitable for nutraceutical delivery.

The riboflavin concentration in the gastric compartment during digestion of zein microspheres was lower ($p < 0.05$) than was obtained during digestion of SPI microspheres or with the control (free riboflavin) run, as shown in **Figure 3a**. The 2.1×10^{-2} mg/mL detected at 10 min increased to 2.7×10^{-2} mg/mL at 30 min. Riboflavin in the duodenum compartment followed the same trend, but at lower concentrations ($p < 0.05$). It is noted that the riboflavin concentration in the jejunum compartment was much lower [$(1.8-4.3) \times 10^{-3}$ mg/mL) than was the case with SPI microspheres ($p < 0.05$) and that the concentrations in the jejunum and ileum compartments were similar throughout the run. Riboflavin availability for absorption from pure zein microspheres (**Figure 3b**) was slower than from the pure SPI microspheres within 1 h of digestion ($p < 0.05$), but still rather quick. The riboflavin found in the jejunum and ileum dialysis fluids after 1.5 h was 63% of the total load introduced into the TIM-1. The availability for absorption curve then began to level off, with 71.8 and 18.8% of the riboflavin being detected in the jejunum and ileum dialysis fluids at the 4 h point, 3.4% in the discharge from the ileum, and 6% remaining in the small intestinal compartments. The increased rates of riboflavin availability for absorption from the jejunum and ileum compartments both followed first-order kinetics ($r^2 \geq 0.996$). The higher riboflavin concentration in the gastric compartment than in the duodenum compartment in the case of zein microspheres suggests that release occurred mainly in the simulated stomach. Low riboflavin concentration in the jejunum compartment suggests relatively slower core release than from SPI microspheres, because once released, most riboflavin could be absorbed, whereas for SPI microspheres the riboflavin was quickly released to rush into the jejunum compartment before absorption. However, core release from zein microspheres was still quite fast, because the nutrient showed up quickly in the jejunum dialysis fluid, which was not expected. According to our previous dissolution data, riboflavin was released slowly into simulated GI tract buffer, resulting in only 20−30% release after 4 h. The highly crystalline structure and hydrophobic nature of the zein microspheres explain this slow release rate (*19*). These factors no doubt prevented permeation of the medium into the zein matrix, slowing the subsequent dissolving and release of the riboflavin. The calculated diffusion coefficients for zein microspheres were much lower (1.9×10^{-9} and 3.5×10^{-9} cm^2/s at pH 1.2 and 7.4 buffer, respectively) than for SPI microspheres. However, using the TIM-1 system, both SPI and zein microspheres showed quick riboflavin release in the GI tract. Hurtado-López et al. reported that zein particles remained spherical in shape and no visible surface porosity and/or roughness was observed, even after 1 week of incubation in buffers at pH 2 and 5 (*36*). However, they underwent degradation in the presence of pepsin and pancreatin in simulated gastric and intestinal fluids. It may therefore be presumed that zein microspheres were rapidly degraded by the pepsin, trypsin, and pancreatin secreted in the TIM-1 apparatus, resulting in quick release of the riboflavin for absorption. This rapid degradation may be attributed to the conformation of the zein molecules in the microspheres. When forming a water/oil emulsion, the hydrophobic portion of protein molecules tends to interact with the oil phase, turning hydrophilic side chains toward the inside of the matrix. The resulting particles are more susceptible to hydrolytic attack by pepsin, because this protease preferentially attacks peptide bonds involving hydrophobic aromatic amino acids (*37*). Although diffusion contributed to riboflavin release to some extent, release due to diffusion seemed to be much slower than that due to protein degradation. Matrix degradation should therefore play a major role in regulating riboflavin release from zein microspheres. The increased rate of riboflavin availability for absorption in both the jejunum and ileum compartments followed first-order kinetics, similar to SPI microspheres, indicating that the limiting step in nutrient uptake is still absorption. The quick breakdown of zein microspheres in the stomach compartment makes them no better suited than SPI for nutrient delivery applications.

SPI−zein complex micropheres gave riboflavin concentration and availability for absorption profiles that differed substantially from the previous three examples. Riboflavin concentration was very low (1.9×10^{-4} mg/mL) in both the stomach and jejunum compartments at the 10 min point (**Figure 4a**), then increased steadily and reached a maximum ($\sim 2.4 \times 10^{-2}$ mg/mL) at 45 and 60 min, respectively, in the stomach and duodenum compartments, after which the duodenal concentration gradually decreased. It should be noted that riboflavin concentration in the duodenum compartment was significantly higher ($p < 0.05$) than in the gastric compartment during the first 30 min. The concentration measured in the jejunum compartment at 30 min (7×10^{-3} mg/mL) was significantly lower ($p < 0.05$) than in the case of pure SPI, but slightly higher than that of zein microspheres ($p < 0.05$). This concentration reached a maximum at 45 min and then decreased slowly. Riboflavin concentration was very low in the ileum compartment ($\leq 1.5 \times 10^{-3}$ mg/mL). The profiles of the riboflavin availability for absorption from SPI−zein complex microspheres (**Figure 4b**) showed a steady increase over the 4 h period in both the jejunum and ileum compartments, reaching 68.9 and 13.1%, respectively. The discharge from the ileum compartment contained 2.3% of the total riboflavin load, whereas 15.7% riboflavin remained in the small intestinal compartments. The increased rate of riboflavin availability for absorption in the simulated gut followed near zero-order kinetics ($r^2 \geq 0.986$). These results indicate that riboflavin concentration and the availability for absorption profile in the simulated GI tract were modified using SPI−zein complex microspheres. The gradual rise in riboflavin concentration in the gastric, duodenum, and jejunum compartments within 45−60 min and the steady increase in absorption during the whole test period indicate that riboflavin was released slowly from these microspheres. Significantly higher riboflavin concentration in the duodenum compartment than in the gastric compartment during the first 30 min suggests that the SPI−zein complex microspheres survived gastric acid and pepsin attack to a large extent, thus delivering encapsulated riboflavin to the duodenum compartment for postgastric release. The duodenum compartment appeared to be a major site for nutrient release. The relatively high riboflavin concentration in the jejunum compartment at 45−60 min suggests that some microspheres reached the simulated jejunum intact for release and direct absorption of the payload. However, it is difficult to calculate the exact amount released in each compartment of the TIM-1. Upon reaching the jejunum, the released riboflavin should be absorbed quickly, resulting in low riboflavin concentration in the ileum compartment. Although synthetic polymers have been developed as gastric coating materials to protect drugs in the stomach compartment and allow them to reach the small intestine intact for absorption, they consist of cellulose derivatives and methacrylic acid copolymers that are not considered to be safe for daily consumption. Natural polymers that are safe for food use, such as alginate and soy protein, generally provide poor barriers against gastric secretions. The interesting property of

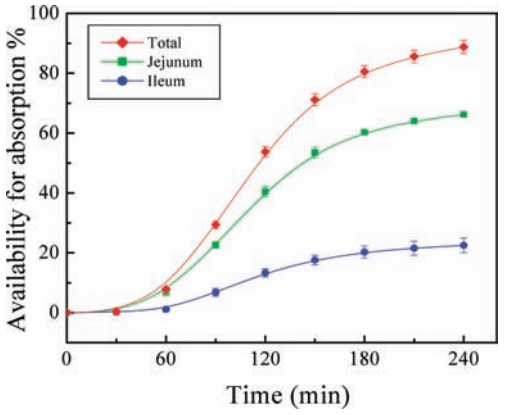

Figure 5. Riboflavin availability for absorption profiles in the TIM-1 gastrointestinal tract compartments after feeding riboflavin-loaded SPI−zein complex microspheres suspended in yogurt. The values are means ± SD (n = 3).

SPI−zein complex microspheres in delivering core ingredients to the small intestine for gradual release and absorption should therefore lead to their application as delivery vehicles for sensitive nutrients. The near-zero-order kinetics of riboflavin availability for absorption from microspheres indicates that the limiting step in nutrient uptake would be release from the dosage form after oral administration (a zero-order core release model in this case) rather than nutrient absorption (*20*). It should be emphasized that near-zero-order absorption would favor maximal utilization of a nutraceutical product in the human body. This sustained release can be attributed both to diffusion and to the protein complex matrix breakdown mechanism. Compared to pure zein microspheres, SPI−zein complex microspheres seemed to break down much less in the gastric compartment. This may be the result of a less hydrophobic surface due to the presence of SPI and hence less vulnerability to attack by pepsin. The same surface presents no obstacle to breakdown by trypsin and pancreatin beginning in the duodenum compartment, thus allowing riboflavin release for absorption.

Prandial State. Because the nutrient release properties of SPI−zein complex microspheres appeared to be desirable under fasting conditions, they were examined using the TIM-1 under prandial conditions. The availability for absorption of riboflavin from these microspheres suspended in a mixture of yogurt and saline solution followed an "S" curve, featuring a conspicuous delay with barely 10% of the total riboflavin in the jejunum and ileum fluids during the first hour, as shown in **Figure 5**. The riboflavin availability for absorption then increased steadily with time and began to level off after 3 h. Riboflavin absorbed from the jejunum and ileum compartments at the 4 h point reached respectively 66.3 and 22.5% of the total load, whereas the discharge from the ileum contained 5.4 and 5.8% remaining in the small intestinal compartments. Over 50% of the availability for absorption followed near-zero-order kinetics in the simulated gut in the prandial state after about 2 h. The conspicuous delay in riboflavin availability for absorption during the first hour of digestion of SPI−zein complex microspheres suspended in yogurt may be attributed to slower riboflavin diffusion in semiliquid food than in water and decreased protein matrix degradation rate in the presence of other food ingredients (milk protein in this case). The rate of nutrient availability for absorption is often slower when ingested with food, compared to the fasting condition. Such effects could be important for allowing nutrients that are sensitive to gastric pH and pepsin to reach the intestine intact for absorption. The near-zero-order kinetics of the availability for absorption for > 50% riboflavin suggests that the SPI−zein complex microspheres can provide sustained release of encapsulated riboflavin over a period of 4 h under prandial conditions. These interesting properties under prandial conditions further demonstrate the application potential for these SPI−zein complex microspheres.

The use of in vitro ADS methods to study nutrient availability for absorption in the simulated GI tract with or without food has been very limited so far. In summary, the present work demonstrates that SPI−zein complex microspheres can provide sustainable release of riboflavin within 4 h after oral administration and near-zero nutrient absorption profiles in both the fasting and prandial states. Incorporation of these microspheres into yogurt can delay riboflavin release after oral administration, which could be desirable, because this would increase their likelihood of reaching the intestine for absorption in an intact and active condition. These SPI−zein complex microspheres therefore show potential for use as delivery systems for nutraceuticals such as riboflavin to create new functional foods (e.g., yogurt enriched with vitamins).

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