Discover Food

Review

A review of in-vitro digestibility models on diverse foods in various segments of human digestive tract

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Abstract
In order to simulate the process of food digestion in the gastrointestinal (GI) tract, various in-vitro digestion models have been developed. These models vary from simple, single-compartment systems to more complex setups with multiple compartments and dynamic characteristics. In-vitro models have mostly been employed to analyze the structural changes and release of food components during digestion in different simulated gastrointestinal environments. The results obtained from in-vitro models of digestion differ significantly from those of in-vivo models because it is difficult to accurately replicate the highly intricate physiological and physiochemical processes occurring in the human digestive tract. The rate and location of food digestion in the gastrointestinal tract is considered very important for human health. However, this feat cannot often be realized for technical, ethical, or budgetary reasons. The significance of in-vitro models lies in their ability to provide reproducibility, the flexibility to select a controlled environment, and the simplicity of sampling. In-vitro models serve as valuable tools for conducting mechanistic investigations and testing hypotheses. This review provides a concise overview of in-vitro digestion models utilized for studying the digestion of different complex compounds that can predict the level of digestibility for a variety of foods. Digestibility models differ in predictability of digestibility. While some of them are designed more for controlled nutrient breakdown assessment, others more realistically mimic real life dynamic digestion.

Keywords Dynamic models · Gastro-intestinal tract · In-vitro digestion · Static models

1 Introduction

There is a growing interest within the scientific community to gain a deeper understanding of how dietary intake impacts human health. One method of filling knowledge gaps involves concentrating on and analysing the meal while it is digested in the gastro-intestinal tract. To analyse the behaviour of food in the gut, in-vitro techniques that mimic digestion processes are commonly used (Guerra et al. 2018). Human nutritional research, the current "gold standard" for dealing with diet-related issues, is complemented by in-vitro procedures, which present advantages in

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terms of expediency, affordability, reduced labour intensity and ethical flexibility. This capability facilitates concurrent screening of a substantial sample population. In vitro models serve as invaluable instruments for the conduct of mechanistic investigations and the formulation of hypotheses, owing to their inherent reproducibility, adaptability in the selection of controlled experimental parameters, and the convenient facilitation of sampling at the designated location of interest [26, 75, 76].

In-vitro models of digestion are frequently utilized to explore changes in structure, assess digestibility and examine the release of food constituents within simulated gastro-intestinal environments. Nonetheless, disparities frequently arise between the outcomes acquired from in-vitro models and those observed in in-vivo models. These discrepancies stem from the inherent difficulties in accurately replicating the highly intricate physiological and physiochemical processes taking place in the human digestive tract. These models are often employed to examine the digestibility, structural alterations, and release of food contents within simulated GI conditions. Nonetheless, the outcomes obtained from in-vitro models of digestion often differ from those observed in in-vivo models, primarily because of the difficulties in accurately simulating the highly intricate physiochemical and physiological processes that take place within the human digestive system [96]. The majority of the food samples evaluated, according to in-vivo digestive models, were made up of plants, meat, fish, milk products, and emulsion-based foods. To make in-vivo models better, measurements could be taken at each stage of digestion rather than basing everything off of final blood glucose levels. This is how sampling breakdown products along the digestive tract will allow for specific tracking of nutrient digestion over time. More advanced tools like non-invasive imaging and even specialized sensors and micro-sampling could perfect this approach and give a clearer view of the specific digestion process for particular foods and link stages of digestion into final indicators such as blood glucose [71]. Digestive enzymes were the most often employed bile in the digestion models.

Using in-vivo models to study food digestion is fairly expensive, complicated, and occasionally unethical. In-vitro digestion models are laboratory systems simulating food break-down in the human digestive system. They are used in nutrition, food sciences, and pharmacy research for studying the liberation of nutrients and bioavailability of active ingredients, as well as effects of digestions without any need for experimentation on humans or animals [17]. The use of in-vitro and in-vivo models can explain how starch is broken down and absorbed in the body. In vitro models feature enzymatic breakdown of starch into glucose; thus, they mimic the action of salivary amylase and pancreatic enzymes. The models are currently in use for the measurement of release rates of glucose, predictions of the glycemic index, and assessment of starch digestibility under controlled conditions. In-vivo starch digestion is impacted through gastric emptying, intestinal motility, and enzyme activities. The blood glucose levels that are measured following starch ingestion in an animal model can be verified and then refined to the best predictions that emanated from in-vitro studies. Through cross reference between the two approaches, scientists manage to understand how the food composition, such as the amount of dietary fiber, affects starch digestion and its effects on the human body [94]. In-vitro models of protein digestion reproduce this process with controlled conditions and enzyme conditions using enzymes, such as pepsin and trypsin, in digestion of proteins into peptides and amino acids. Static models retain fixed conditions; dynamic models, however, mimic real-life variabilities—such as varying enzyme activity and food movement in the case of digestion processes. The application and use of these models help researchers understand the process of protein breakdown and rates of nutrient release, thus their bioaccessibility. However, in-vivo digestion is an individual-based process dependent on subjects' gastric acid secretion and gut microbiota or health conditions. Although in-vitro models are useful, they are far from perfect when the entire complexity of protein absorption within a living organism is concerned. To perfect such models, one typically compares in vitro data with in-vivo data so that these models may be much more precise in predicting real digestive outcomes [33, 101].

Several models of in-vitro have been found to examine the gastro-intestinal behaviour of food or medications, in-vitro techniques emulating digestion processes are frequently used. Because of their reproducibility, versatility in selecting a controlled environment, and simplicity of sampling, in-vitro models are great instruments for mechanistic investigations and hypothesis-building [26]. Drugs, mycotoxin, and lipids have all been investigated using human digestion static in-vitro models to ascertain their digestibility and bioaccessibility (i.e., the volume of a chemical that is thought to be liberated from the matrix and made available for absorption via the gut wall). Furthermore, these models have been utilized to investigate the liberation of secondary plant compounds, such as carotenoids and polyphenols, along with micronutrients, like minerals and trace elements, from the plant matrix [16]. Understanding the physicochemical alterations that take place in food during digestion as well as the various elements affecting nutrient bio accessibility, bioavailability (the overall quantity of a substance that is released and absorbed in order to reach the blood stream, which distributes it to the tissues), and other properties is essential.

Designing functional food products would benefit from having knowledge of the recommended daily nutrient intake, the processing methods that would amplify the health advantages of bioactive compounds, and digestibility, which is defined as the percentage of food constituents that are converted into available form and is present in the complete digestible, soluble, and non-soluble fractions [3, 68]. It is evident that the digestive system is at the centre of many problems that have been addressed in a variety of sectors, including nutrition, toxicology, pharmacology, and microbiology, not only by researchers but also by commercial enterprises [36, 67]. However, utilizing animal models as a substitute should also be eschewed as much as possible because researching the intricate process of digestion in humans is challenging, expensive, individual, and constrained by ethical considerations. Hence, scientists have developed and employed in-vitro models to simulate the digestive system of humans for research purposes. Digestive enzymes were the most often employed bio-molecules in the digestion models. The consideration of how food behaves in the digestive tract is a crucial aspect of designing both modern-day processed foods and food products derived from plants and animals that generate complete foods and food ingredients [72]. It is well understood how human physiology, chemistry, and biology work together to process various dietary components in the typical human gastro-intestinal system. The chemistry of digestion was the focus of early studies, more recently, there has been a growing emphasis on investigating the breakdown of semi-solid and solid foods, leading to a more comprehensive study of the microstructural and mechanical elements of the process [98].

The numerous researches on in-vitro gastro-intestinal digestion systems have revealed how matrix and components of matrix behave during digestion process. Its use has evolved throughout time, both in terms of the process requirements (including parameters, length scale, protocols and guidance) and the choice of new application domains [61]. For instance, the most significant advancements over the past ten years have concentrated on standardizing and harmonizing static and dynamic in-vitro models that simulate gastro-intestinal (GI) activities by outlining crucial parameters and settings that can be used for various purposes and facilitate cross-comparison of research findings across different research teams, studying the structural aspects of the food matrix, antioxidant properties and bio accessibility of various bioactive compounds. Brodkorb et al. [9] standardized INFOGEST in-vitro digestion models by working on the consistency and reliability of numerous food digestion studies. INFOGEST helps to standardize pH levels, enzyme levels, and digestion times for each stage of digestion, giving researchers a chance to replicate any study conducted worldwide and thus compare results. Standardization has become the gold standard for gaining more accurate insights into digestion in food, nutrient bioavailability, and nutrient release across all food types. These models have shown to be useful research tools to better comprehend the behavior of a food during digestion. Despite the complexity of the human gastro intestinal system, research in food and nutrition using IVD (In Vitro Digestion) models has become active and very promising, allowing us to make significant advancements in our comprehension of digestive proteolysis and lipolysis in human digestion [66]. However, since these simulations cannot completely replace In vivo trials, conclusions and interpretations from such studies should be used with caution [14, 97]. The review demonstrates the significance of in-vitro digestion models. The models have rapid and reproducible tests that enable one to compare the patterns of digestion and determine how digestible food is. But despite the fact that the models provide controlled conditions that can be replicated, they cannot fully simulate the complex process of digestion within the body. They also cannot provide accurate predictions for digestibility.

The novelty of this review is that it further advances the knowledge about digestion by improving in-vitro models to closely mimic the particular digestive processes of the different populations and health conditions. This study is different from previous ones in that it will examine more closely the mechanisms of food disintegration based on the types of matrices involved, like soft cereals versus hard foods, and on different physiological conditions, like pH and temperature, through focusing attention on integration of mass, heat, and momentum transfer in analysis. This stresses the harmonization of key parameters such as pH and enzyme activity and provides population-specific models, for instance, infants, the elderly, or cystic fibrosis patients-to support personalized nutrition and to different health needs [119].

2 In-vitro static/dynamic digestion models

Recent years have seen the development of various in-vitro models of digestion that vary from static mano-compartmental to multi-compartmental dynamic structures [120]. The majority of outdated attempts are made to study how food is digested using static equipment such as a pH–stat, agitated water bath, air bath rotation and other items through the use of a number of stirring containers to replicate a single set of biochemical variables in each

compartment of the gastro-intestinal tract. These static models imitate the conditions of either the stomach or the intestine. However, the enormous variety in how various individual models use digestive parameters is a key challenge for these methods [99]. The primary parameters include pH, the length of each phase of digestion, the quantity and the type of digestive enzymes utilized, the rate of stirring or agitation, the quantity of food samples, and others. These differences make it strenuous to compare the results of contrasting research organizations and draw general conclusions. For instance, the oral and stomach stages were included in a well-known in-vitro static digestibility model published by Englyst, Kingman, and Cummings [99]. A conventional static in-vitro digestion methodology, aligned with the latest knowledge of in-vivo digestive settings, was published [28]. A mincer was used to imitate the mastication process according to the INFOGEST consensus protocol. A polypropylene centrifuge tube was positioned within a spinning wheel mixer, maintained at an operating temperature of 37 °C, to imitate the ongoing motions and rhythmic peristaltic contractions characteristic of the upper gastro-intestinal tract in humans. The oral, gastric, and intestinal digestive enzymes were composed of amylase salivary, pepsin and pancreatin. Sample aliquots were taken at varied incubation times for the upcoming digested micronutrient assay. While in-vitro static models are commonly employed to evaluate the digestibility of various food constituents, they simplify gastro-intestinal physiology and do not accurately replicate the dynamic facets of digestion, specifically fluid dynamics and mechanical forces that take place in a series of solid beakers with constant stirring. In order to create hypotheses and conduct mechanistic research, static models are typically utilized, with particular usefulness. These models have been used to address diverse scientific concepts about bioaccessibility of pharmaceuticals, mycotoxins and macronutrients such as proteins, carbohydrates and lipids. They have also been used to study matrix release of micronutrients such as minerals and trace elements, and secondary plant compounds including carotenoids and polyphenols. Some digestion methods are used to produce bioaccessible fractions that can be used to address further mechanistic questions, such as intestinal transport by employing Caco-2 cells [7].

In-vitro dynamic digestive models (Fig. 1), including mano-compartmental and multi-compartmental structures, have become increasingly complex in recent years. As a result, dynamic models, as opposed to static ones, can represent the mobility of digested food, continuous discharges of digestive fluids, fluctuating concentrations of enzymes, and progressive pH alterations over time that take place in in-vivo circumstances. The (Simulator of the Human Intestinal Microbial ecosystem-SHIME), (TNO gastro-intestinal model-TIM), (Dynamic gastro-intestinal model-DGM), and other (Gastric digestion simulator-GDS) are a few examples of dynamic in-vitro systems [30]. In vitro models like that of Caco-2 cells are important for the study of health benefits derived from foods, as they simulate human intestinal conditions. The ability of these components to interact with gastrointestinal cells, protect against oxidative stress, and possibly reduce pro-inflammatory cytokines such as IL-8 is evaluated. Rapid screening testing of extracts will allow the assessment of optimal dosages and times for therapeutic effect, and accelerate analysis of allergies and inflammation. In general, they would provide critical enlightenment as to health-promoting properties of food components that could open avenues for dietary intervention [83].

Numerous gastro-intestinal structures, from static mano-compartmental structures to multi-compartmental and dynamic structures, have been created to represent the process of digesting food. Additionally, certain aspects vary amongst in-vitro digestive models. The quantity and type of stages that go into the digestion process are one of these variables. Simulated digestion models may include the oral, gastric, or/and small intestinal phases, as well as large intestine fermentation in some cases, relying on the investigation's objectives [55]. There are various significant differences between models, such as the chemical composition of the digestive solutions employed during different phases, their types and quantities of enzymes, the salts and buffers utilized, the surface-active substances, the biological polymers, etc. Mechanical stresses also vary at each stage and comprise the fluid flow used at each digestion stage, the flow profile and geometries, the amount and direction of stress applied, etc., are another important variable components [45]. This merely provides a tiny glimpse of the level of intricacy that can be attained with these systems, which, in addition to the variety of conditions that may be used, aid in understanding the challenges associated with comparing the outcomes of different investigations. Static in-vitro digestion is the most straightforward and is generally simple and affordable to utilize on a regular basis. One approach for these models involves employing a single-compartment test that sequentially simulates the oral, gastric, and small intestinal phases [91]. Static models have set parameters, as a result, the ratio of food to secretions, pH levels, and concentrations of enzyme/biosurfactant are predetermined at the start of each phase and remain consistent throughout. These fixed numbers are out to be a best guess average of the GI scenario that is physiologically realistic [95]. Static models, which rely on simplified digestive physiology, do not fully replicate the dynamic aspects of the process of digestion. These dynamic elements encompass mechanical forces, fluid dynamics, continuous secretions, pH variations, and stomach

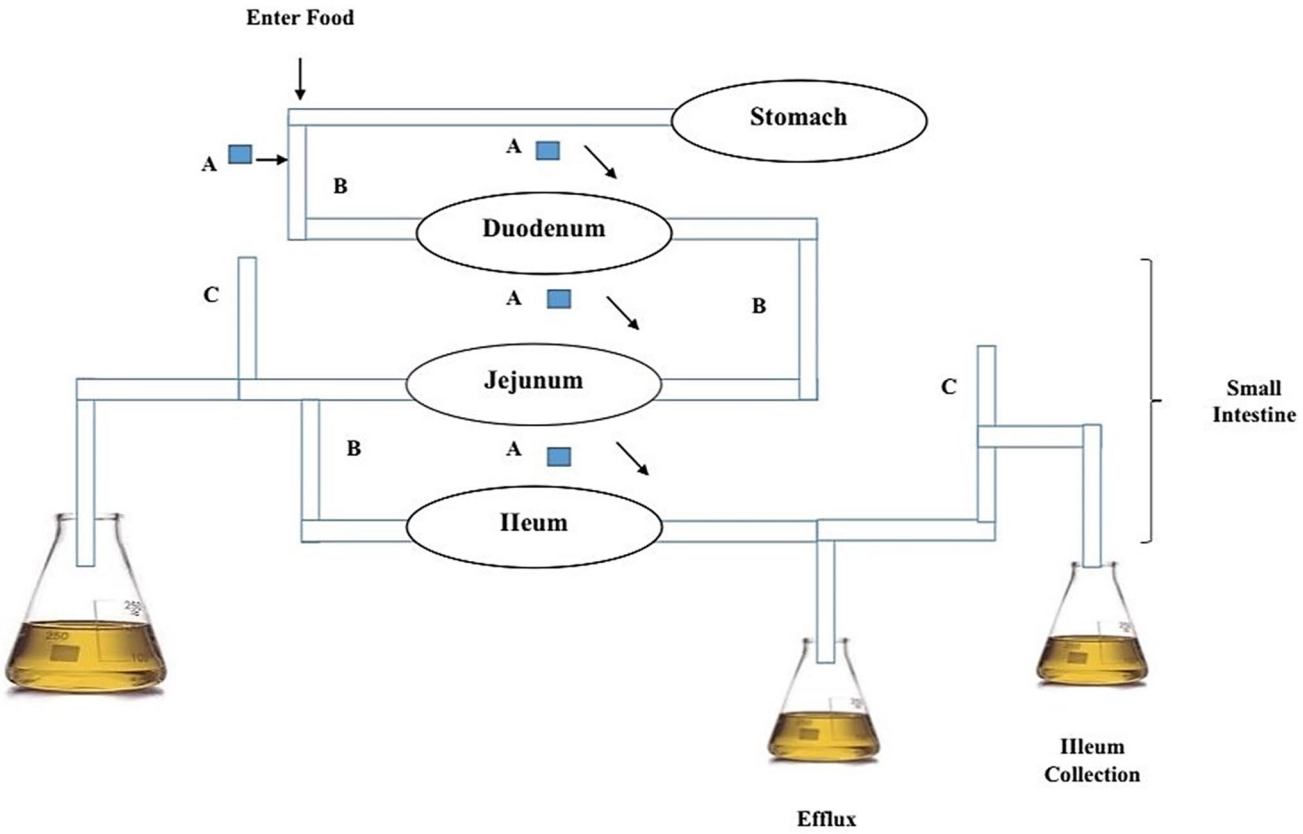

Fig. 1 Diagram of the in vitro dynamic model of the gastrointestinal (GI) tract, including the stomach, small intestine, and large intestine, under simulation. Part **A**, known as the Stomach Compartment, the stomach phase of digestion is simulated, which contains dynamic pH variations, pepsin-like enzyme activity, and mechanical movement such as peristalsis. Part **B**, mimics the duodenal and jejunal phases with regulated pH, enzyme activity like pancreatic enzymes and bile salts, and absorption mechanisms. Drug dissolution and nutrient bioaccessibility can be studied in this compartment. Part **C**, or Large Intestine Compartment, represents the colonic phase and deals with pH gradients, microbial fermentation, and the synthesis of short-chain fatty acids (SCFAs)

emptying, all of which are simulated with constant stirring. Static models are primarily employed for mechanistic research and hypothesis development, serving specific screening purposes. Several static designs have been created and utilized for very specific goals in the past [119] (Bourlieu et al. 2014). The benefits of static models are digestive models should be as straightforward as feasible because they simplify reality. This also holds true for creating model systems or substances that behave in the gastro-intestinal tract. A digestive model needs to encompass all the essential information to forecast the desired outcome. The range of application and complexity increase as the relevant parameter set increases. Static models inadequately represent real enzyme–substrate ratios, pH variations, transit durations, and elimination of digested substances in both time and place, thus limiting their capacity to accurately predict in-vivo bioaccessibility, particularly in terms of gut wall absorption. If the conditions are appropriate for the range of product features, ranking the digestion of various items is more practical [58]. Static models can also be valuable in mechanistic research, which tries to understand how a substrate is digested under particular conditions. The complexity and difference in the composition of a matrix of the various products should be kept to a minimum. In simpler terms, static models are helpful for researching the specifics of how basic meals or single substrates are digested [119].

2.1 Types of in-vitro models involved in digestion

In-vitro digestibility systems, which aim to mimic human digestion and are typically constructed from glass containers, operate under static conditions. These systems lack the capacity to replicate the mechanical forces and dynamic environments that food experiences within the digestive tract. However, when assessing various experimental scenarios and handling a substantial sample size, dynamic setups are better suited to closely resemble in-vivo conditions

[29]. Important aspects of food digestion include the transit of digested products, pH profiles, and substrate-enzyme ratios. Consequently, in-vitro static structures that lack mechanical and dynamic movements impair our capacity to accurately predict nutrient availability or food behaviour throughout digestion. Various dynamic systems have been created to emulate the physiological circumstances of the human digestive system with the repeatability required for scientific investigations (Table 1). These systems are available in both single- or multi-compartmental varieties [58]. These dynamic systems face the difficulty of competing between biological significance and technical sophistication. Most multi-compartmental systems feature compartments for the stomach and small intestine, but very few have spaces for the colon. Dynamic models can continually replicate microbial fermentation, enzyme secretion, peristaltic pressures, and pH change [58]. Not every dynamic in-vitro model perfectly replicates the kinetic, chemical, and mechanical physiological conditions of the digestive system. Only a small number replicate complete mechanical, dynamic, and chemical circumstances, some solely model chemical conditions, yet others concentrate exclusively on mechanical conditions [98, 111].

2.1.1 TNO gastro-intestinal model (TIM)

A dynamic in-vitro system that replicates the human upper gastro-intestinal tract is the TNO in-vitro gastro-intestinal digestion model (TIM-1). This model considers a number of factors, including peristaltic mixing, bile addition, pancreatic digesting enzyme addition, and passive absorption [108]. TIM is a computer-controlled, dynamic, and multi-compartmental system designed to replicate the functions of the digestive system. The system's design concentrated on the primary physiological traits that change with place and time. These characteristics include contractions, transit duration, pH levels, composition, the rate of secretion of digestive juice, and the absorption of nutrients and water [5, 98]. Computer simulations employed the protocols created with accurate in-vivo data in the system. There are specialised protocols that may be established based on factors like age, health and dietary type. A widely used dynamic in-vitro system in food and pharmaceutical research to analyze how nutrients and medicinal ingredients are released and absorbed is TIM [24, 116]. The TNO dynamic in-vitro model of the colon, controlled by a computer, was made by TNO some 15 years ago. It is the outgrowth of the TIM-1 system (the TNO in vitro gastrointestinal model of stomach and small intestine). In summary, the model consists of four interconnected glass reactors, with one flexible membrane positioned in between. Between the glass jacket and the membrane, water is maintained at body temperature (37 °C for humans, 39 °C for pigs, 41 °C for birds, etc.). The temperature is controlled through a temperature sensor. There are a number of features in TIM-2 that are different from other models. Firstly, the peristaltic movements of the flexible membrane provide better mixing and movement of components throughout the entire model than would be achieved by stirring (in a fermenter) or shaking (on a rocking platform or otherwise). In TIM-2, there is no phase separation of solids and liquids, which does occur in other systems. Viscous 'meals' or insoluble components can be used without issues in the system. Because of its strong mimicry of physiological parameters, experiments in TIM-2 are usually conducted for one week. Other models that mimic the large intestine usually take several weeks to stabilize [112]. Additionally, the microbial inoculum can be tailored to suit specific target groups, e.g., children, elderly, or specific patient groups. The pooled microbiota used in the model is demonstrated to represent a standardized microbiota, allowing a series of experiments from an identical starting point. The colonic environment is mimicked by managing pH, temperature, and low oxygen conditions. The model also includes a dialysis system to avoid metabolite buildup, thus maintaining metabolite concentrations at a physiological level. Samples can be taken from both the lumen and the dialysate, giving information on the microbiome and metabolites. This model has been tested and is routinely used to study bacterial communities, and can be used to study fungi or fungal–bacterial interactions as well [63]. The advantages of the TIM system include its capacity to faithfully reproduce the dynamic physiological conditions of the GI tract, its capacity to handle particular food components, medications and complete meals, its applicability across a wide range of research areas in both food and pharmaceutical studies and are not restricted to any one application, and the ability to collect samples from various sources. For various age groups, they may replicate usual GI circumstances, biological variation, and illness states. The tests are remarkably repeatable due to rigorous control of all parameters and the composition of secretory fluids during the analysis of chyme transit, which provides precise insights into the fate of test items in the GI tract [43]. Since there is no intestinal mucosa, intestinal cell lines or tissues must also be examined in order to examine absorption. The absence of feedback on the connection between a meal's energy density and GI conditions, which must be pre-set in the TIM software, is another drawback of TIM systems. Instead of measuring bioavailability, which takes metabolism and excretion into consideration, it is

Table 1 Types of in-vitro models involved in digestion

In-vitro digestion model	Study	Food compound	Application/objectives	References
Static model	Useful for a brief investigation on digestion. Dynamic features of the digestive process, such as mechanical forces, fluid dynamics, continuous secretions, PH gradients or gastric emptying in a series of stiff vessels under constant stirring, are not adequately modelled by static models, which oversimplify digestive physiology	Food (starch, protein, lipid rich) in this model is utilised by using homogenised simple isolated containing compound	Applied to prevent starch resistance, lipid and protein breakdown. Enhance food qualities; preliminary studies to support conceivable nutrition and health claims. As a result, the static models are mostly employed for mechanistic investigations and the development of hypotheses, with specialised applicability for screening purposes	[54, 95]
SHIME (simulator of human intestinal microbial ecosystem	Created to simulate the gastro-intestinal tract's microbial environment. To model the metabolic fate of food, faecal microbiota is used. SHIME makes it possible to precisely replicate mucosal microbial colonisation. The gastro-intestinaltract's microbial ecosystem has been modelled using the simulator of the human intestinal microbial ecosystem (SHIME)	The source of food (carbohydrate, protein, fat, prebiotics) can be both homogenised and complex for analysis	Provides a way to quantify the degree to which alterations in the microbiome's composition, metabolites of microbes, signalling molecules, or antigens have various consequences on gut barrier of host in terms of permeability and inflammation -related metrics. The device, which has five stages of reactors, mimics the regions of the small intestine, large intestine, and stomach all at once. The initial two reactors employ the fill and draw technique to imitate the small intestine's digestive process and the stomach's acidity and pepsin digestion	[21, 27]
Dynamic in-vitro human stomach system	The study aimed to create stomach empty ingrate for the solid and liquid fraction while simulating the pylorus's human filtering condition	The DIDGI technique has been used to examine a variety of solid and liquid matrices for digestion (such as beef stew with orange juice, cooked rice)	The solid and liquid fractions' stomach emptying rates were calculated using an exponential model. While the solid fractions displayed a delay or lag phase, the liquid fractions did not exhibit such a lag phase	[113]
Dynamic model	Applicable to investigation on complete digestion. Compared to the more comprehensive dynamic models, which should be tested for their capacity to mimic gut conditions	Several compartments are present to perform the food (meat, vegetable, cereal products) analysis	Applied to protein digestion, lipid-digestion, and peptide digestion. Effect of food structure on nutrient delivery, interactions, probiotic survival	[32, 66]

Table 1 (continued)

In-vitro digestion model	Study	Food compound	Application/objectives	References
TNO gastro-intestinal Model (TIM)	Used to examine the human digestion process also mimics human digestion. They provide an exact simulation of the GI's changing physiological circumstances. They are capable of handling both specialised medications and culinary additives. For various age groups, they can replicate typical GI circumstances, biological variation, and illness states	The system was designed to accurately and precisely imitate the primary physiological parameters that vary with time and matrix, such as pH, contractions, composition, transit time, and rate of secretion of digestive juices, captivation of water, and nutrients	It replicates small intestine absorption, variations in peristaltic movement, stomach pH, gastric emptying rates, intestinal transit periods, gastric, biliary, and pancreatic secretion, as well as those organs' functions. The TIM system is sufficient to mimic the changing physiological GI conditions accurately and can succeed in managing specific food items, drugs, and whole meals	[77]
Simulator of gastro-intestinal tract	To simulate fermentation in the colon and gastro-intestinal digestion, this dynamic simulator was developed Moreover, it serves to replicate the colonic microbiota that the large intestine uses for metabolic bioconversions. Also, this system is more significant in the probiotics, nutrition, and health fields The stomach, small intestine, and the ascending, transverse, and descending colons are all represented by separate compartments in the single model, which has five total. This system also regulates the food's flow rate	Foods (probiotics, prebiotics dairy products etc.) both simple and complicated are involved in the study. Food evaluation can be done using complicated and homogenised food components	Five compartments that correspond to the stomach, small intestine, and the upward, longitudinally, and declining colon make up the digestive system The temperature, pressure, and rate of flow of the secretions from the stomach are controlled by computer software to keep them at the desired levels. The intestinal microbiota's polyphenol metabolism is observed using the SIMGI by two participants	[25]
PH stat lipolysis model	The most used in-vitro digestive model for evaluating lipid-based medication delivery strategies. the technique for calculating the rate of lipolysis of lipids in a test tube	Digestive enzymes are manually added to begin the digestion process, replicating the addition of pancreatic juice. The porcine pancreatic ex. contains pancreatic lipase and other esterase	The model will have a reasonably straightforward and affordable setup that may be utilised for LbDDS (lipid-based drug delivery system) screening. The model depicts a (lipid base drug delivery system) LbDDS screening setup that is quite straightforward and affordable	[89]
in-vitro mechanical gastric system (IMGS)	To discover how more accurate stomach peristalsis would affect the intestinal lipid breakdown of protein-stabilised O/W emulsions. It has been demonstrated that the ultimate degree of lipolysis, which underlies protein-stabilised O/W emulsions is significantly influenced by the long-term stability of the O/W emulsion	In this gastric system the food (Oil-in-water (O/W) emulsions) is composed of semi solid to soli	IMGS model is able to produce peristaltic movement as stomach of humans, with propulsion and retro propulsion and griding by enhancing moving frequency of four pair piston	[91]

Table 1 (continued)

In-vitro digestion model	Study	Food compound	Application/objectives	References
Engineered stomach and small intestine	In vitro human data have been used to validate the model for pharmacological applications. A prolonged release form of theophylline and an immediate release variant of paracetamol were examined as model medicines. Theophylline and paracetamol levels in the dialysis samples were tested	Model created to enable nitrogen flushing under anaerobic circumstances and inoculation using human faeces samples	So, at this point only therapeutic uses requiring liquid digestion have been permitted for its usage. To verify the approach during the digestion of solid foods and for nutritional or microbiological applications, further validation studies are required	[41]
Dynamic digestion gastro intestinal tract (DIDGI)	In this system computer simulation created with data from in-vitro observations regulates the temperature, pH, meal flow rate, secretions produced by the digestive system, and emptying rate for each step. This work demonstrated that the DIDGI might be used to gain some insight into the kinetics of how human milk is digested. The effects of treatment with heat on the rate at which proteins of milk hydrolyse and release peptides	Different matrices of sample are used to accomplished the analysis obtained by this system. The food products used in this study were transglutaminase-induced acid gel (TG) and rennet-induced gel (RG)	The DIDGI has mostly been used to research how dairy ingredients and products are digested. Some studies have concentrated on the investigation of the protein and fat digestion of human milk and new born milk formula because it was first created with the infant's digestion in mind	[59]
The dynamic gastric model (DGM)	A model capable of physiologically accurate simulation of the mechanical and biochemical processes involved in human gastric digesting. The factors such as nutrient bio accessibility, how food structure affects nutrient delivery, how nutrients interact, and how functional foods survive and are delivered	Normal and complex foods (meats, vegetables, grains) are involved in analysis. Homogenised and complex food products can be used for the analysis of the food	Food simulates the physical forces involved in mixing, transportation, and breakdown within the normal physiological range when consumed in this way. The system alters the duration of the stomach stay, the quantity and pace of adding enzymes and acid and the physical processing, which depends on the meal matrix	[105, 120]
Human gastric simulator (HGS)	Gastric digesting is the system's primary objective. HGS is used to look at the chemical and physical degradation of food and other substances in the stomach using physiologically relevant properties	The system analysis is based on the food(water, glycerol, Aqueous Xanthan Gum Solutions) that is generally intake and studied in live	The drawbacks include the need to continue validating the combining and physical property modifications of sample meals using in vivo data The oral and small intestine stages of digestion are not included	[48]

Table 1 (continued)

In-vitro digestion model	Study	Food compound	Application/objectives	References
Dynamic gastric model (DGM)	Intricate and precise feeding matrices, like to those used in in vivo research, can be used using the approach. The rate of nutrients released from stomach digestion has been frequently utilised to evaluate the rate of hydrolysis and breadth of macronutrients and bioactive substances in the GI upper tract. Additionally, the DGM has been utilised to investigate probiotic GI tract survival	The equipment facilitates the usage of intricate and precise food matrices(cereal based food, protein rich food, dairy products)	The impact of food processing and matrix has mostly been studied. In order to learn more about the effects of the structure of food and nutrient interactions, microscopy has also been used to analyse the structural changes that occur during digestion. The model's drawbacks include the stomach compartment's vertical arrangement,	[100]
Multi compartmental models	The models that were utilised to represent the stomach and small intestine are discussed in this section	The system analysis revolves around solid food (high in protein) matrices	The GI tract's dynamics are simulated in multiple compartments using multi-compartmental models. Models include TIM, DIDGI comes under multi compartmental models	[18]

feasible to measure bioaccessibility by combining TIM with in silico modelling. The TIM system is a widely recognised and efficient in-vitro method that saves both time and money. It is a reliable means of evaluating the bioavailability of nutrients, the digestibility of food, and the efficacy of functional ingredients. This assessment is conducted under simulated dynamic conditions resembling those of the human adult and infant gastro-intestinal systems [58]. The bioaccessibility of macronutrients, both fat- and water-soluble vitamins, bio-active substances and minerals has been successfully studied using the TIM [24]. A nutrient's ability to be assimilated depends on its properties, the structure and meal matrix composition and the physical reaction of the human body to both the nutrient and the meal as they traverse the gastro-intestinal tract. TIM does not encompass feedback mechanisms related to meal-specific details, a limitation shared by other digestive models. To address this, the TNO Nutrition and Food Research Institute in the Netherlands has engineered a computer-controlled, dynamic, multi-compartmental system meticulously designed to emulate the in vitro conditions of the GI tract of humans as well as those of monogastric animals [43].

2.1.2 Simulator of the human intestinal microbial ecosystem (SHIME)

The microbial environment of the digestive tract has been modelled using the simulator of the human intestinal microbial ecosystem (SHIME) (Fig. 2). Together, the stomach, small intestine, and sections of the large intestine are simulated by the system's five reactor phases [58]. The initial two reactors employ fill-and-draw method to mimic the stomach's acidity and pepsin digestion as well as the small intestine's digestive process. Peristaltic pumps are utilized for precise control over the movement of digestive fluids and vessel contents. The final three vessels served as models for the ascending, descending and transverse colons and they were continuously agitated with a magnetic stirrer. It is possible to regulate the pH levels and the transit time of the contents within the vessels to simulate in-vivo data. To ensure anaerobic conditions, the temperature of the system is kept at 37 °C, and a daily flush of nitrogen (N2) is carried out for 15 min [27] using faecal microorganisms to replicate the metabolic processes of food. The advantages of utilizing SHIME technology as a basis for the experimental purpose include the capacity to work with volumes comparable to those found In vivo, the presence of two to four complete gastro-intestinal tracts in the single system (i.e., TWINSHIME to Quad SHIME), and the capacity to culture the microbiota of the intestines in different sections of the colon for extended periods, often spanning several months [19]. The enabled investigations using a repeated daily dozing technique to assess how the microbiota activity and composition have changed in response to a particular treatment, additionally, the Mucosal-SHIME (M-SHIME

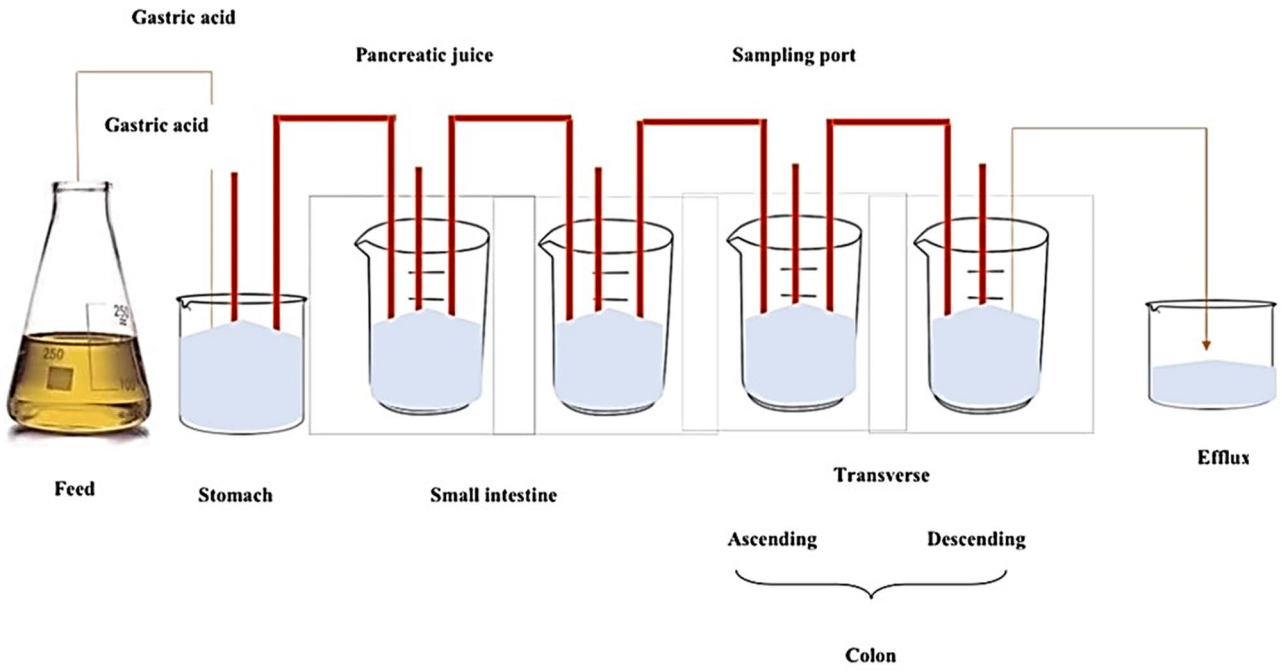

Fig. 2 Model of simulator of the human intestinal microbial ecosystem (SHIME). It consists of connected bioreactors that mimic the stomach, small intestine, and colon, allowing the controlled study of gut microbial interactions and metabolic processes relevant to human health

simulate the colonization of mucosal microbes. As the mucosal microbiome is in close proximity to the host's epithelial cells, it is believed to have a more intrinsic potential to affect human and gastro-intestinal health [38]. The SHIME (Fig. 2) modular design enables the investigation of the inter-individual variation in microbiome behaviour in response to particular therapies. Direct contact with the epithelial cells of the host is possible using colon suspension [58]. Therefore, the degree to which changes in the composition of the microbiota, microbial metabolites, signalling molecules, or antigens modulate the gut barrier permeability and inflammation-related parameters of the host might be assessed [2]. The SHIME, like all in-vitro simulators, is hampered by the lack of a physiological environment. Additionally, metabolite absorption and water are not commonly replicated in the intestinal compartment.

2.1.3 Dynamic digestion gastro intestinal model (DIDGI)

The dynamic digestion system known as INRA, developed by the French National Institute for Agricultural Research (located in Rennes, France), employs two glass-covered tanks to replicate the functions of the stomach and small intestine. These tanks are equipped with temperature-controlled water baths that facilitate the injection of water into their jackets (Elie et al. 2019). To mimic the sifting function of the human pylorus, a Teflon membrane having 2 mm perforations is positioned between the stomach and intestinal compartments, just before the transfer pump. The computer simulation system, informed by in-vivo observations, rigorously regulates various parameters, including temperature, pH levels, food flow rate, digestive secretions, and discharge rates within each compartment [8] (Deglaire et al. 2016). Despite the DIDGI method being used to digest a variety of matrices (including dairy, meat, fruits, vegetables, and emulsions), only information on the digestion of cheese and infant formula was acquired [20, 29]. The in-vivo and in-vitro digestion of newborn formula were compared to show that this method was physiologically relevant. The newborn formula for which the in-vivo trial was done was fed to 18 pigs at a higher concentration of lipids and proteins than the usual formula but at the same ratio of lipids to proteins. Utilizing cutting-edge technology, the improved infant formula underwent in-vitro gastro-intestinal digestion, and the amount of milk protein breakdown was monitored and compared to the outcomes observed in live subjects [39, 78]. To replicate the digestive conditions of elderly people, Adouard et al. [1] conducted a study using a modified dynamic in-vitro model of digestion DIDGI to mimic the older digestive parameters as accurately as possible. The DIDGI dynamic In vitro digestion was performed on the three experimental formulations (F1/F2/F3) over the course of a 4 h duration using parameters catered to the elderly. These formulations varied in composition and method of application. The protein breakdown and lipid breakdown rates of these formulations were contrasted. The extent of proteolysis at the conclusion of the digestion process was little impacted by the procedure (liquid vs spray-dried), with 50.8% for F2 compared to 56.8% for F1 and 52.9% for F3, with 5% of the variation among these formulations. In terms of the level of lipid breakdown, the inclusion of bovine cream resulted in 63.7% and 60.2% for F2 and F3, respectively, as opposed to 66.3% for F1 (which contained only vegetable oil).

2.1.4 Simulator of gastro-intestinal tract

The Institute of Food Science Research in Madrid, Spain, designed this dynamic simulator, to imitate gastro-intestinal digestion and colonic fermentation. The SIMGI (Stimulator of Gastro-intestinal tract) model (Amigo et al. 2018) has five compartments that collectively represent the stomach, small intestine, and ascending, descending, and transverse colon (Fig. 3). In the area of the stomach, a flexible silicone container is enclosed by two transparent, rigid plastic vessels. To mimic peristaltic motions, water at a temperature of 37 °C is forced through the gap between the plastic modules and into the flexible container [4, 93]. Computer software modifies the digestive content flow rates, temperature, and pressure levels to match the specified parameters. The small intestine, the climbing, horizontal, and declining colon are the other four compartments, and they all function as continually stirred reactors with regulated pH and anaerobic environments. Each compartment of the system has a collecting point where samples can be collected for biochemical and microbiological examination. Peristalsis and chyme transit behaviour are combined in this system for the stomach and small intestine compartments [93]. Akter et al. [62] studied food digestion within gastro-intestinal tract by employing silver nanoparticles within a dynamic stimulator of the gastro-intestinal system. The simulation of gut-microbial digestion was first carried out in an in-vitro static model utilising two types of silver nanoparticles: solid polyethene glycol-stabilised silver nanoparticles (PEG-AgNPs 20) and liquid glutathione-stabilised silver nanoparticles (GSH-AgNPs). Based on these tests, a dynamic model (SIMGI) that reproduced the stomach, small intestine, and ascending, descending and transverse colon in physiological settings was used to mimic the digestion of GSH-AgNPs. GSH-AgNPs dynamic transport in the SIMGI, was comparable to that shown for the inert substance Cr-EDTA, which eliminated any changes in intestinal fluid

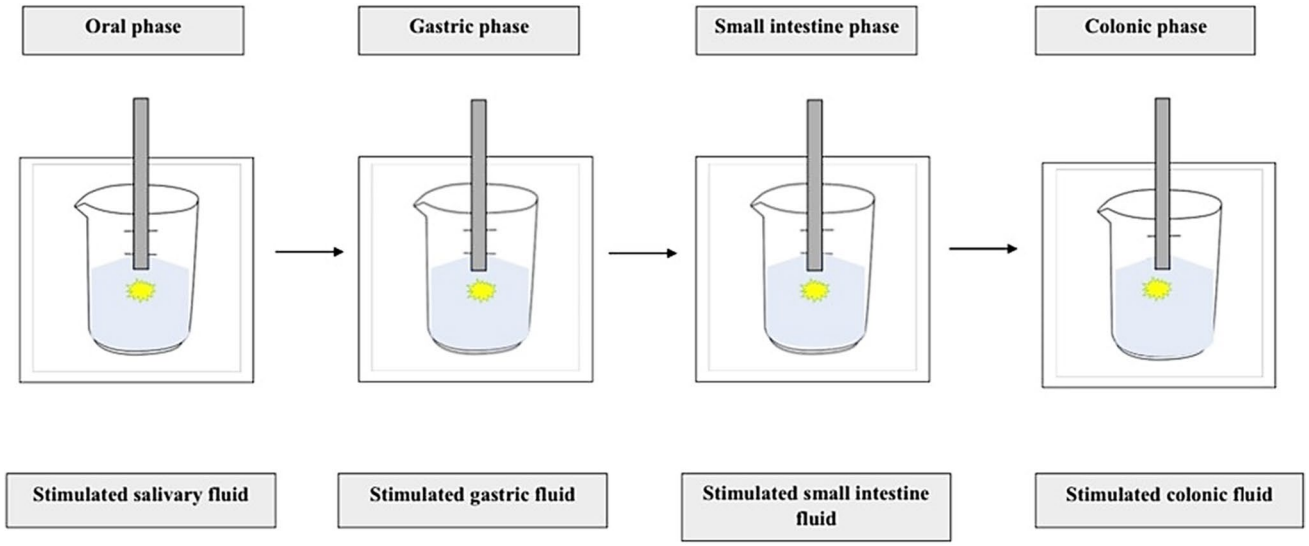

Fig. 3 Simulator of gastro-intestinal tract. It includes sequential bioreactors that simulate the stomach, small intestine, and colon, enabling detailed studies of digestion, microbial activity, and the effects of various substances on gut health

delivery brought on by the AgNPs. Additionally, feeding GSH-AgNPs to the SIMGI model did not lead to notable alterations in the composition or metabolic activity, particularly protein breakdown activity, of the gut microbiota.

2.1.5 Dynamic in-vitro human stomach (DIVHS)

The Suzhou Key Laboratory of Green Chemical Engineering at Soochow University developed an advanced model known as the Dynamic in-vitro Human Stomach (DIVHS). This enhanced version builds upon earlier models like DIVRS-I, DIVRS-II, and RD-IV-HSM (China), as described in [113] study. The new DIVHS has a temperature-controlled box, a driving mechanism, a secretion and emptying system, and a vessel for the stomach and duodenam. Using 3D printing technology, a soft elastic silicone vessel in the form of a J shape with dimensions resembling those of the human stomach is produced. Contraction is simulated via a system of rollers, eccentric wheels, and motors. A unique roller-added mechanism enhances sieving and disintegration capabilities. The tilting angles of an auxiliary emptying device were utilized in controlling the rate at which stomach emptying rates for solid fraction had a lag phase, while liquid fraction did not were determined using an exponential model [6]. Bao et al. [44] studied the impact of structural differences between brown and white rice on gastric emptying, and starch digestion was examined in this work using a dynamic In vitro human stomach system. During digestion, changes in starch hydrolysis, pH, viscosity, and gastric digesta particle size distribution were examined. Brown rice displayed higher pH buffering capacity, larger digesta particle size, and greater rheology due to the outer bran layer's protective effect against structural degradation. This delayed the process of gastric emptying and resulted in reduced starch hydrolysis and overall digestibility of starch in the stomach when compared to white rice. This research has offered quantitative proof of the significance of macrostructural factors, specifically the bran layer, in the gastric digestion of rice, which may also have an impact on intestinal absorption.

2.1.6 Gastric digestion simulator (GDS)

The Food Research Institute of Japan has recently developed a human gastric digestion simulator (GDS) which tries reproducing the antrum's functions. The antrum is also trapezoid-shaped with constriction towards the pylorus. Besides having a gastric section, GDS also possesses a roller mechanism, a section for temperature control, and a panoramic view of the actual food disintegration through transparent windows [50]. Features such as a mounted camera enable particle scrutiny for up to 180 min. This group also developed a continuous GDS (c-GDS) that adds gastric secretion and emptying systems [51]. Chao Zhong et al. [12] made use of this simulator to observe and quantify digestion during the gastric phase of digestion (they were considering the simulator as a fundamental device for observation and quantitative study of these processes). The system supports chemical and environmental conditions within the simulator without compressing physical emulation of stomach digestion, which is reproduction of peristaltic movement—an essential

process for mechanically reducing solid food materials into smaller fragments. The GDS was effectively deployed to directly monitor and assess the breakdown process of Tofu (bean curd), which serves as a representative solid food rich in protein. The investigation centred on the characterization of the distribution of size and content of protein of Tofu particles throughout the course of digestion experiments. The findings elucidated noticeable distinctions in particle disintegration dynamics when compared to conventional flask shaking experiments.

2.1.7 pH stat lipolysis models

The pH–stat lipolysis model illustrated in Fig. 4 is one of the most popular in-vitro digestion models designed to assess lipid based drug delivery systems (LbDDS). This model represents a system for (Liposomes based drug delivery systems) screening which is simple and relatively economical. Usually, a single compartment is used in the experimental arrangement to simulate the intestinal digestion [89]. During the in-vitro digestion process, a reaction vessel with temperature control is used.

Within this vessel, the tested LbDDS is dispersed in a digestion medium that has been carefully formulated to mimic the conditions found in the duodenum. This includes appropriate pH levels, concentrations of bile salts (BSs) and phospholipids (PLs), and buffer capacity to replicate the effect of secretion of bile. This digestion medium is similar to intestinal fluid found in either a fasted or fed state. Digestive enzymes are manually added to begin the digestion process, replicating the addition of pancreatic juice. In contrast, though pig pancreatic extract is one of the widely used sources of pancreatic lipase, and has been shown to be quite similar to the human pancreatic extract, the porcine pancreatic extract esterase hydrolyzes triglycerides and other digestible LbDDS excipients to free FAs [102]. Quantity of sodium hydroxide (NaOH) needed to neutralize the pH to its original state after enzymatic hydrolysis that will essentially serve as the proxy for the digestion level. In order to precipitate the FAs formed in digestion, calcium is either continually administered during the digestion experiment (the dynamic lipolysis model) or given as an early bolus (the static lipolysis model). FAs will accumulate at the emulsion interface and obstruct further digestion if they are not removed from the digestive media [69].

2.1.8 Colonic model

Different types of in-vitro colonic models exist, such as straight-forward batch faecal incubation with a reliable and completely anaerobic faecal microbiota, which are ideal for metabolic studies, and more complex continuous systems with one or more parts pH-controlled resembling human colon segments, or in-vitro dynamic gastro-intestinal colonic structure models [60]. In-vitro colonic models have two major limitations. Firstly, they are incapable of accurately recreating

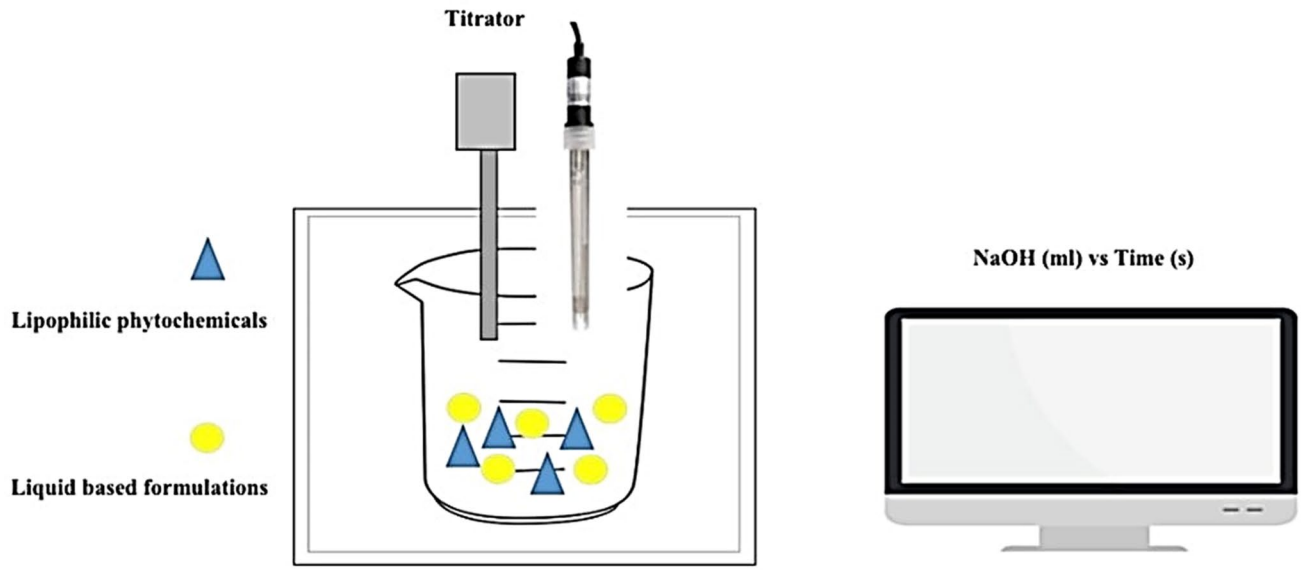

Fig. 4 pH stat lipolysis models. These models maintain a constant pH by automatically titrating acids or bases, mimicking the natural pH environment of the digestive tract. This setup allows precise monitoring of lipid breakdown and enzyme activity, facilitating the analysis of lipid digestion kinetics and the effects of various dietary components

the in-vivo rates of catabolism and absorption. Secondly, there are inaccuracies associated with the representation of bacteria within the intestinal lumen and mucosa. Static or batch models are employed in the assessment of differing chemical sources or doses and are vital for preliminary analysis of metabolism of colonic phenolic compounds that is likely to be high due to substantial inter-subject variability. The microbial population in these simulations is intended to be repeatable, particular to the colon, and suitable for in-vivo conditions. Furthermore, it needs to be stable after inoculation. Static or batch models are used to assess different sources or dosages of the metabolism of phenolic complexes in the colon, which may be influenced by inter-individual variability.

For the long research necessary to test colonic microbiota's geographic and temporal response to dietary phenolic compounds and the metabolism of microbes of these photochemical, dynamic, multi-compartment colonic models are helpful [80]. These simulations are designed to include a reproducible microbial population that ought to be consistent after inoculation, specific to the colon, and appropriate to in-vivo conditions. In vitro studies should simulate intestinal absorption in order to effectively eliminate waste products generated through microbial metabolism, thereby preventing any adverse effects on the colonic microbiota. Unfortunately, the dearth of research on the development of microbial biofilms that adhere to the colonic epithelium limits the ability of colonic models to replicate the in-vivo conditions. The circumstances found in human or animal colons are replicated by a one-stage fermentation model known as ARCOL (Artificial colon). A paradigm that enables fermentation to maintain anaerobiosis only through the metabolic processes of the microbiota without the need for flushing with nitrogen or carbon dioxide has been created for the first time. The method considers variables, including pH, temperature, anaerobic conditions, the existence of a complex, the presence of replicated ileal effluents, colonic residency time, metabolically active microbiota, and the passive absorption of water, high-density and microbial metabolites that are significant for in-vivo fermentation in the large intestine [81]. One of the few wireless colonics in-vitro models, ARCOL is equipped with dialysis fibre to simulate passive absorption of microbial products and can maintain anaerobic conditions through the specific activities of the intestinal microbiota. The impact of a single or repeated treatment of drugs of interest on the makeup and activity of the intestinal microbiota can be evaluated using the ARCOL (Artificial Colon) model [25, 92].

2.1.8.1 MICODE MICODE (Multi-unit In vitro colon gut model for Digestion and fermentation Experiments) was employed for testing the prebiotic effect of fiber blend with D-Limonene supplement (FLS) compared to fructooligosaccharides (FOS) in Nissen et al. [84]. This model enabled the analysis of the volatilome and central microbiota dynamics during fermentation which allowed the assessment of the biological impact of the supplement on gut microbiota and metabolite formation [84]. MICODE, an in-vitro model of the distal colon, is specifically designed for simulating human colon fermentation. Earlier, controls pertaining to the microbiota and the volatilome were incorporated into MICODE to increase stability of the system. It can maintain throughout the fermentation process the original human microbial diversity, including the racial and ethnic groups preserved in stool samples, alongside some Archaea and over 400 operational taxonomic units (OTUs). Application of multivariate statistics in conjunction with MICODE, a robust yet versatile model, was effective for formulating a methodology detailing effects caused by alcalase hydrolysis and elucidating prebiotic potential within hydrolysates [85].

2.1.8.2 Micro matrix The micro-Matrix bioreactor system is a state-of-the-art *ex-vivo* colon model that tries to solve the problems of throughput and experiment time of current models. This system's ability to process 24 samples in 48 h makes it ideal for screening a wide range of samples [70]. The micro-Matrix platform facilitates the *ex-vivo* distal colon experiments process optimization on a stepwise basis. But one thing that has been mostly commonly reported in this model is the event of *Escherichia coli* blooms. The system provides guideline procedures to reduce these *E. coli* blooms that can tamper with the experiments' precision [70]. Although the micro-Matrix system offers advantages with regard to throughput and timeline, it should also be noted that other 3D in-vitro colon models have been created that better match the complexity of the native tissue. For instance, 3D bioprinting methods have been used to fabricate perfusable tubular models through the use of tissue-specific biomaterials such as colon-derived decellularized extracellular matrix (Colon dECM) [42]. These more complex models could provide further information on tissue development and disease progression that the micro-Matrix system may not be able to capture. In summary, the micro-Matrix bioreactor system represents a major step forward in ex-vivo colon modelling, with high-throughput capabilities and shorter experiment times. Although it overcomes some of the limitations of current models, researchers should take the particular requirements of their research into account when choosing among various in vitro colon models since more advanced 3D systems will be necessary for some purposes [42]. The micro-Matrix a 24-well sophisticated parallel controlled cassette-based bioreactors as a batch colon model.

The machine can operate 24 individual fermentations simultaneously and are relatively cost effective. Based on next generation sequencing analysis, the micro-bioreactors offer a high degree of reproducibility together with high-throughput capacity. This makes it a potential system for large screening projects that can then be scaled up to large fermenters or human/animal in-vivo experiments. Benefits of mini-bioreactors especially the micro-Matrix include integrated online monitoring and parallel control of fermentations using integrated sensors. Each well of the micro-Matrix cassette operates as a stand-alone bioreactor allowing for cassette-wide gradients of pH, dissolved oxygen and temperature. The pre-calibrated integrated pH and DO sensors remove the need to calibrate each individual well prior to beginning the fermentation which even with a smaller number of bioreactors is time difficult and time consuming. The benefit of the micro-Matrix cassette (used in this study) is that, up to 24 different conditions can be tested at any time since each cassette house can be inoculated with faecal slurry and maintained under physiological conditions [87].

2.1.8.3 The Smallest Intestine in-vitro model (TSI) The Smallest Intestine in vitro model, or The Smallest Intestine (TSI), is a small in vitro system to increase throughput by mimicking the transit through the stomach and small intestine (SI).

The core TSI module contains five reactors, each with a working volume of 12 mL. In the simulated SI passage, the bile is absorbed, and pH is set to physiologically relevant levels for the duodenum, jejunum, and ileum. A consortium of seven representative bacterial members of the ileum microbiota is also included in the ileal stage of the model. TSI allows for the screening of numerous samples at low cost and within a short period, thus constituting a suitable in-vitro screening platform [15].

3 Application of in-vitro digestibility models

Most research has been done on how medications and macronutrients (mostly proteins) behave during digestion. The three most significant ones encompass the influence of digestion on the availability of bioactive substances and their antioxidant capabilities, the specific influence of the food matrix, with a primary focus on dietary fibre in vegetable-based foods, on these attributes, and the impact of digestion on coating integrity (particularly in nano-delivery systems for bioactive compounds). However, new application areas have emerged in the recent ten years (2000–2016). It has uses in several branches of science, including food science, microbiology, pharmacology and nutrition [61, 74]. Toxicological studies, drug testing, tailored therapy, regenerative medicine, fundamental life science, and tissue engineering are just a few of the fields that use in-vitro models. During in-vitro digestion, the involvement of food properties, enzyme type, and enzyme concentrations are important regulators of food digestion [56]. The status of protein sources, digestion circumstances, proteolytic enzyme impacts and differences in digestibility (raw versus processed). Increased dietary protein intake causes a rise in pancreatic proteolytic enzyme production, whereas increasing starch or fat intake causes a rise in amylase and lipase secretions, respectively [11]. Therefore, while adjusting in-vitro digestion parameters like digesting duration, enzyme concentrations, or enzyme composition, sample attributes must be taken into account. For example, when target substance concentration (such as carbohydrate, lipid, or protein) is raised, adjustments are required in the concentration of enzymes or the duration of digestion, even if all other aspects of the in-vitro digestion process remain constant [115]. However, Green et al. [40] observed that the inclusion of digestive enzymes did not result in any significant difference in the extraction of catechin from green tea when employing an in-vitro model of digestion. They observed that the quantity of catechin recovered through the in-vitro digestion model incorporating digestive enzymes was equivalent to that previously reported in a method that did not utilize enzymes. This might be because plant-based diets are difficult for persons with mono-gastric stomachs to digest, therefore whether or not there were any enzymes present had little effect on catechin release.

4 Digestion by lipases, proteases and amylases

In-vitro digestion models depend on a variety of key factors that encompass sample properties, enzyme activity, ion composition, mechanical forces, and digestion time that generally govern macronutrient degradation—lipids, proteins, and carbohydrates, as well as bioactive compounds' release. The several digestive enzymes are lipase, protease, and amylase that play specific roles for the nutrient digestion process.

The in-vitro digestion is considered to depend on the parameters of the sample properties, enzyme activity, ion composition, mechanical forces, and digestion time. Lipases for instance are enzymes that catalyze the hydrolysis of lipids, which include phospholipids, fat-soluble vitamins, and cholesterol esters. Unlike esterases which operate in water, lipases are only active once they have become adsorbed to an oil/water interface. This lipase performs critical functions in the digestion, transportation, and processing of dietary lipids in most organisms [65]. PNLIP (Pancreatic Lipase) is the principal enzyme that digests ingested fats, however, it needs bile salts and colipase to be activated and perform its function. Like all other enzymes, pancreatic lipase has conditions under which it works best and bile salts create such conditions. Bile salts are secreted from the liver, are stored in the gallbladder, and are released during a meal. In addition to being secreted, bile salts have the ability to inhibit the activity of the pancreatic lipase enzyme by using the pancreatic lipase alone and isolating it from the lipid-water membrane. A small cofactor protein known as colipase, which is secreted by the pancreas, neutralizes this effect of inhibition by binding to the pancreatic lipase and securing it to the surface of the lipid droplet, thus, restoring the enzymatic reaction [121]. The hydrolysis of triglycerides into free fatty acids and monoglycerides, which are further taken in by the intestinal epithelium, can only happen with these three components, colipase, bile salts, and pancreatic lipase [64]. Pancreatic lipase, unlike gastric lipase, which starts lard digestion in the belly, is reliant on bile salts and colipase for functioning. This dependency is especially important in vitro digestion models, for example the INFOGEST protocol that replicates the basic human gastrointestinal conditions [117]. The INFOGEST model also adds bile salts to simulate the processes of emulsification and enzymatic fat digestion to the level of pancreatic lipase activity [103]. This is critical when studying lard digestion and bioavailability in nutrition and food science. Further studies should focus on enhancing these models to adequately capture the interactions of pancreatic lipase, colipase, and bile salts in cases of lipo malabsorption disorders and treatments.

As a protease, an enzyme that cuts peptide bonds responsible for protein decomposition into smaller peptides and amino acids, is considered one of the most important catalysers to aid in nutrition digestion by providing physical and chemical alterations for the assimilation of proteins [52]. Different types of Metallo and Serine enzymes are known to exist as inactive zymogens, which undergo a myriad of structural transformations until they reach their final pro-enzymatic form. This metamorphosis allows traces of pepsinogen and trypsinogen to exist in the cells of the intestines or stomach. Active forms such as trypsin and chymotrypsin make use of ischemic zymogen activation, enabling their adept specific proteolysis functions within the intestine. The rest of the active zymogens can only work in the pro-enzymatic structure of cheif cells and the body of the stomach allowing them to carry out hydrolysis with precise specificity. Hence why proteases cut peptide bonds at such a specific assured level [53, 104].

The 60–70% of the total caloric input provided by the starches consists of amylose and amylopectin, the glucose polymers with linear and branched linkage [57]. While granules of starch are insoluble and would presumably not interact with taste receptors, heat treatment above the gelatinization temperature swells granules, causes crystallinity loss in them, and leaches them into solution. While solubilized starch is too big to bind the taste receptors, mastication and salivary α-amylase action, which ruptures α-1,4 linkages, breaks it up into smaller-sized glucose oligomers like maltose and maltotriose and possibly enables taste perception of the starch [90].

Salivary α-amylase (AMY1) and pancreatic α-amylase (AMY2) are the major isoforms of α-amylase, a key enzyme in carbohydrate digestion, that catalyze the hydrolysis of α-1,4 glycosidic bonds present in starch, leading to the production of shorter oligosaccharides, maltose, and maltotriose. AMY1 is key to energy metabolism by initiating starch breakdown in the oral cavity and continuing this process in the small intestine. Recent advances have underlined the genetic diversity of AMY1 copy numbers, which affects enzymatic activity and is linked with variability in dietary adaptation and metabolic health. In addition, research has explored the influence of α-amylase inhibitors on glycemic regulation and their potential medicinal applications in diabetes and obesity treatment [46, 110]. pH, ion composition, and the availability of co-factors such as calcium, which stabilize its structure, all contribute to the activity of the enzyme. The creation of functional meals and enhancing in vitro digestion models to study nutrient bioavailability and metabolic responses depend on the understanding of the role of α-amylase in the digestion of starch [22].

5 In-vitro digestion of micronutrients and phytochemicals

Apart from macronutrients, a significant range of micronutrients and phytochemicals, or compounds that may potentially offer health benefits, although they are not essential, have been used in-vitro digestion simulations [47]. Phytochemicals, also referred to as secondary plant components, are much larger than micronutrients and comprise a variety of compounds, including phytosterols, triterpenes, polyphenols, carotenoids, glucosinolates, and many more [55, 82]. In-vitro digestion studies have been performed on a number of micronutrients and phytochemicals, encompassing trace elements such as iron and zinc, minerals like magnesium and calcium, carotenoids, a variety of phytosterols, polyphenols, vitamins such as B6, B12, E, and D, and other dietary components (including cholesterol). Recent studies on cereal-based foods relate to the health benefits of grain polyphenols, with emphasis on digestibility and intestinal absorption. Experiments conducted on pigmented grains, including purple rice, barley, and wheat, demonstrated that though the total amount of phenolic decreases after digestion, purple rice maintained the highest percentages, 79%, and antioxidant activity 31%. However, among all the pigmented grains, there was a good preservation in the gastric phase of antioxidant activity, although important compounds were not detectable after intestinal digestion. Indeed, protocatechuic acid, vanillic acid, apigenin, and chrysoeriol all were able to permeate the intestinal barrier. Results that reflect huge differences in terms of composition and activities allow for pathways toward recognition of bioavailability and stability [31]. Levi et al. [99] stated that the primary focus of the study will be on the most abundant water-soluble phytochemicals (polyphenols), the most abundant fat-soluble phytochemicals (carotenoids), and the trace element iron. Additionally, their dietary consumption has been linked to safeguarding against deficiencies of micronutrients, such as iron and vitamin A, as well as preventing chronic diseases, including carotenoids and polyphenols. Dietary fibre-rich matrices found in fruits and vegetables interact with phytochemicals to modify their relative bio accessibility. Courraud Berger's study on vitamin A and carotenoid standards indicated that they were not stable when compared to dietary carotenoids [109]. The carotenoids in food matrices demonstrated a higher level of preservation, with recovery rates ranging from 30 to 100%, in contrast to the standards, which exhibited recovery rates of only 7 to 30%. This was demonstrated in research that contrasted the stability and bioavailability of purified vs whole-food derived carotenoids. Choudhary et al. [13] conducted an investigation on the stability and antioxidant potential of anthocyanins found in fresh red cabbage. The findings indicated that the stability of acylated anthocyanin in red cabbage during in-vitro gastro-intestinal digestion was notably affected by the composition of the food matrix. Panchal, John, Mathai, and Brown [88], Fattore et al [35] proposed that vegetable components, in particular dietary fibre, prevent the unstable anthocyanins from degrading under physiological conditions that replicate the antioxidant properties of anthocyanins found in fresh red cabbage and in its anthocyanin-rich extract in order to assess the impact of the composition of red cabbage. Klunklin et al. [49] stated that the results demonstrated that red cabbage acylated anthocyanin stability under in-vitro gastro-intestinal digestion is significantly influenced by the food matrix. Further, under the simulated physiological conditions, dietary fibre and other components of vegetables shield the labile anthocyanins from degradation. Table 2 represents the in-vitro models used for the evaluation of different analytes.

6 Limitations of in-vitro approach

A laboratory-based in-vitro method may be accurate and reliable, but it is essential to correlate the resulting data with in-vivo data to obtain physiologically meaningful measurement of digestible protein. Although 100% agreement between in-vitro and in-vivo findings may not be realistically expected, in-vitro assays should still be considered valuable resources. The body's ability to digest food proteins can be used to rate them [7, 55]. As our understanding of protein structure in the context of digestion advances, it may be useful for predicting the nutritional value of an organism in-vivo. For some foods, chemical ingredient data and multiple regression models with in-vitro digestibility measurements have been presented. Moreover, the utilization of in-vitro digestion methodologies plays a significant role in advancing our understanding of the mechanisms governing the liberation of amino acids and peptides during the digestion, as well as the impact of protein structure on the nutritional quality of dietary proteins [107]. Typically, the following criteria need to be satisfied when devising in-vitro digestibility assessments: matching in-vivo enzymes in terms of their presence, enzyme–substrate ratios and sequence; standardising specificities and enzyme activities;

Table 2 In-vitro models used for the evaluation of different analytes

Evaluated analyte	Digestion model	Main objective	Main results	Reference
Fructo-oligosaccharide	In-vitro static-digestion models are used	Using pancreatin and porcine bile extract for oral gastric and small intestine digesting	Slight degradation in gf2 can be observed	[86]
Hydrolysed curdlan (1-3-beta-D-glucan oligosaccharides)	In-vitro static (infogest) digestion models	Pancreatin and porcine bile extract are used in oral gastric and small intestine digestion during this process	There is no degradation at any stage	[73]
Citrus pectin	Dynamic (SIGMIE) digestion model	Gastro-intestinal, small intestinal, colon (ascending, transverse, and descending), and bile salts digestion	There is a slight reduction in molecular weight following intestinal digestion	
Polysaccharides from Gracilaria rubra	Static digestion model is used for the screening purposes	Pancreatin and porcine bile extract are used to observe the three phases of oral gastric small intestine digestion	No degradation takes place in digestion	[23]
Polysaccharides from aloe vera	Static in-vitro digestion model is used for analyte analysis	Analyte digest in the small intestine (alpha-amylase)	No degradation takes place	[106]
Starch sample	Static in-vitro model is used to estimate the starch sample	take part in the three stages of oral, gastro-intestinal, and small intestinal digestion (of bile salts, pancreatin, and brush border carbohydrates)	Degradation was reported in this model	[37]
Homogenised and complex sample	SHIME (simulator of the microbial ecosystem in the human intestine)	To investigate the mechanic olfaction of products and ingredients (homogenised and complex food) that had been initiated	Shown varying impact host's gut barrier permeability and inflammation-related metrics	[27]
Dairy, fruits, meat and vegetables, emulsions	Dynamic in-vitro stomach model	For the liquid and solid fractions, gastric emptying rates were calculated using an exponential model	In contrast to the solid fractions. liquid fractions, had a lag phase	[114]
Complete meal, drug and	TNO gastro Intestinal model (TIM)	The system produces the pH levels, transit time composition, and rate of secretion of gastro-intestinal fluids	They can manage individual food components, medications, and full meals	[77]
Homogenised and complex food products (probiotic food)	Simulator of gastro-intestinal tract	A computer software regulates the temperature, pressure, and flow rates of the digestive secretions to maintain the specified levels	Also, this system is more significant in the probiotics, nutrition, and health fields	[79]
Semi solid to solid food	In-vitro mechanical gastric system (IMGS)	To determine how intestinal lipolysis of protein-stabilised O/W emulsions could be impacted by a more realistic stomach peristalsis	IMGS model is able to produce periplastic movement as of the human stomach, with propolusion and retro propulsion and griding	[91]
Protein rich food	Multi compartmental models	This section goes through the models used to simulate the stomach and small intestine	The GI tract's dynamics are simulated in multiple compartments using multi-compartmental models	[18]
Pharmaceutical application	Engineered stomach and small intestine	A prolonged release form of theophylline and an immediate release variant of paracetamol were examined	To test the model throughout the digestion of solid meals and for nutritional or microbiological purposes	[41]

regulating co-enzymes and co-factors, pH levels, and temperature; segregating digested material from undigested components while considering the inhibitory effects of end products on digestion; and accounting for the influence of sample size, particle size, and digestion time. It is practically impossible to mimic the complex physiological processes of digestion and absorption in a laboratory setting [98]. Factors such as anti-nutritional agents, dry matter, dietary fibre, endogenous protein secretions, gut enzyme activity, and gut microbiota cannot be reproduced in a laboratory setting. The sensitivity of an assay depends on the duration of the reaction and the enzyme–substrate ratio. To accurately replicate in-vivo digestion and the release of proteins from the food matrix, in-vitro investigations may necessitate the inclusion of lipases, sugars, and elastases. Although it is assumed that every soluble substance will be digested, some small peptides, particularly those contained in heat-treated proteins, may not be absorbed by the body [118].

7 Conclusions

In conclusion, in-vitro digestion (IVD) models have become indispensable tools for simulating digestion, providing information on bioavailability, structural release and nutrient release. These models, which range from simple static systems to complicated dynamic setups, have helped to progress food science by allowing for controlled, reproducible investigations. However, they cannot entirely imitate human digestion since in-vivo and in-vitro results differ. Standardized methods, such as INFOGEST, give a solid foundation, but there are still gaps, particularly in our understanding of carbohydrate, lipids and complex food matrix structures. Future study should improve IVD models to better match clinical or animal investigations, ensuring their usefulness in designing foods and nutrition.

To be functional in personalized nutrition, IVD models need to be tuned for the physiological conditions, such as diseases of the digestive organs, infants and aged. Additionally, the application could be enhanced by combining IVD with functional assays including nutrient release kinetics or interactions of gut microbiota. However, IVD models would hold tremendous promise for delivering optimized nutritional health without the existing defects that cannot mimic stomach geometry or gastric movements. The gap between food science and nutrition will be closed by continuous attempts to standardize, validate, and modify these models to ensure that they remain accurate, reliable, and sensitive tools for understanding the complex interactions between food and digestion.

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Declarations

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