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Review Article

TIM Systems: A Novel Approach for Determination of Bioaccessibility and Bioavailability of Food Components

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Abstract

The increasing rate of consciousness on innovative changes in foods has been increasingly grew the demand for the information about metabolism of these foods. Data about bioaccessibility and bioavailability of food components become more vital for both consumers and producers in the context of developing new healthier foods. Both nutrition and food researchers try to develop methods that allow user to obtain sufficient results of bioaccessibility and bioavailability of food compounds. Therefore, this study will summarize the TIM system, a novel system that mimics the human gastrointestinal system by all means, including working principles and studies performed in this system. In vivo and in vitro studies cannot be used as useful tool for measuring bioaccessibility and bioavailability of food components because of the physiological differences between human and animal. TIM studies were shown that this novel system could be easily used for the monitoring gastrointestinal movements of foods. In conclusion, TIM systems provide adequate data including digestibility, bioaccessibility and bioavailability of many different food products. By mimicking the GI tract, TIM system is a promising tool for the investigation of digestion, absorption components and health promoting aspects of foods.

Keywords: TNO TIM, bioaccessibility, bioavailability, gastrointestinal, simulation, short-chain fatty acids.

1. Introduction

WHO defines nutrition as 'the intake of food considered in relation to the body's dietary needs'. Nutrition is defined as all of the process includes the steps from how organisms reach nutrients to how metabolize and use them to support themselves. Dietary factors are linked to the prevalence of diseases including obesity, hypertension, cardiovascular diseases etc. Digestion related studies exhibited that digestion and release of nutritional components from foods can be an alternative way for preventing diet-related diseases (Zúñiga and Troncoso 2012). In the recent years, releasing, encapsulation, and protection of bioactive compounds in food matrix become more importance because of their benefit of health (Mc Clements, Decker and Park 2009). However, the benefit of bioactive components affected by many factors as pH, oxidation, digestion, and food matrix. There are many testing systems for bioactivity, bioaccessibility and bioavailability of bioactive compounds in food matrix.

Definition of bioaccessibility can be explained as the amount of food material situated in the gut after releasing from the food matrix, which have the possibility to pass through the intestinal barrier. Bioavailability, on the other hand, is defined as the proportion of the food, which is pass through pathways including digestion, absorption and metabolisation (Rubio et. al., 2014). In vivo and in vitro methods are two main methods to determine the benefit of bioactive compound. When compared *in vivo* and *in vitro* methods, *in vivo* feeding methods always supply the most accurate results but they are expensive and take long time, because of this reason, *in vitro* procedures have been developed (Boisen and Eggum 1991). *In vitro* digestion models are chosen for *in vivo* models because these models present accurate results rapidly. Alternative for *in vivo* methods and it screen food ingredients rapidly (Coles, Moughan and Darragh 2005). Quality of results of animal studies is not adequate because of the huge physiological differences between animals and humans, when a study includes specific forms and effects of foods bioavailability (Verwei et al. 2016).

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A	Stomach Compartment
B	Peristaltic Valve
C	Duodenum Compartment
D	Jejunum Compartment
E	Ileum Compartment
F	Pressure Sensor
G	Stomach Secretion
H	Duodenum Secretion
I	Jejunum Secretion
J	Ileum Secretion
K	Pre-Filter
L	Semi-Permeable Membrane
M	Filtrate Pump
N	pH Electrode
O	Level Sensor
P	Temperature Sensor
Q	Dosing Port

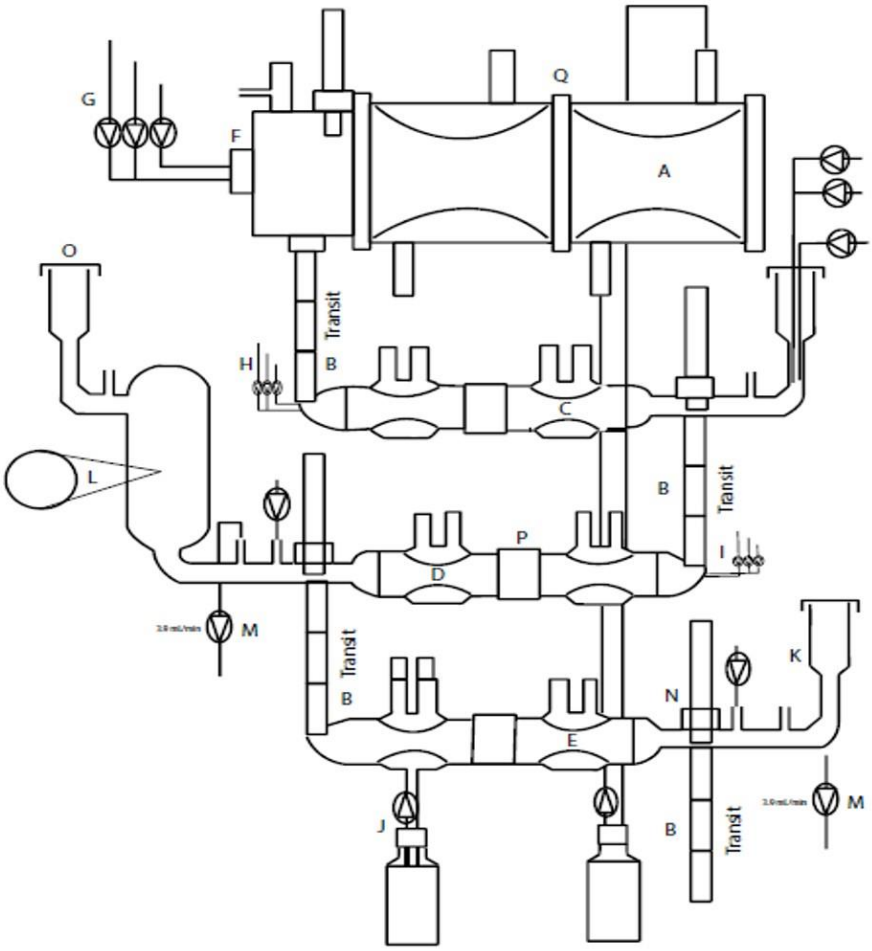

Fig. 1a. Schematic Diagram and Description of TNO TIM 1 System

1	Dialysis Fibers
2	Level Sensor
3	Nitrogen under Pressure/Vacuum
4	pH Electrode
5	Flexible Wall
6	Dialysates
7	Electrolytes
8	Peristaltic Valves
9	Water
10	Pump

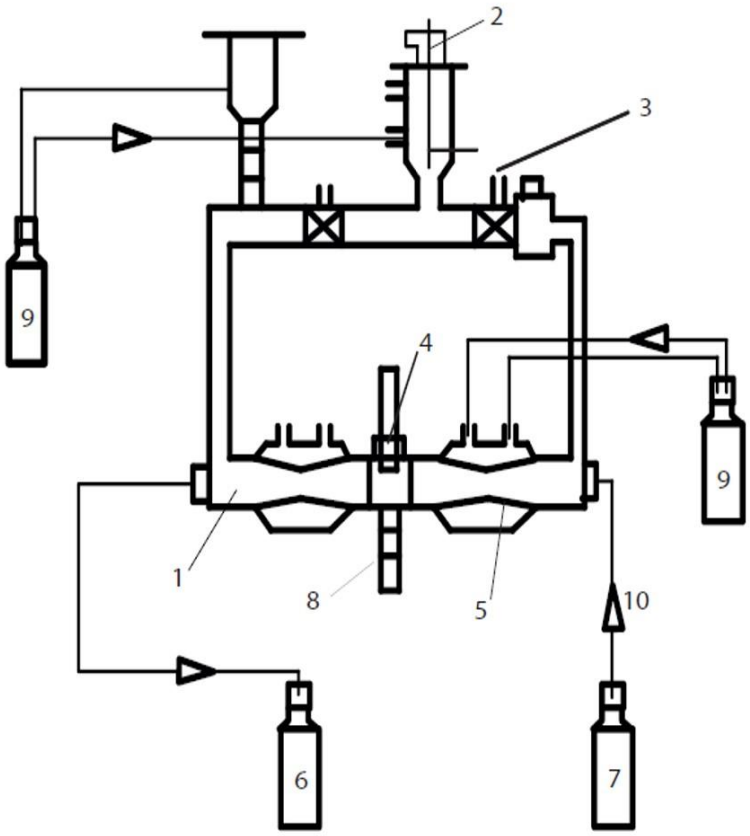

Fig. 1b. Schematic Diagram and Description of TNO TIM 2 System

The TNO Gastro-Intestinal Model is an advanced multi-compartmental (Fig. 1a, 1b) and computer-controlled in vitro model that mimics the human gastrointestinal tract including stomach (TIM-1), small intestine (TIM-1) and colon (TIM-2) (Havenaar, Bellmann, and Zeijdner 2014; Al Hasawi et al. 2017). This system, that allows a dynamic simulation of controlled squeezing, peristaltic movements, absorption of nutrients and water (Gervais et al. 2009), was developed by Department of Physiology, TNO Nutrition and Food Research Institute, Netherlands (Minekus, Marteau, and Havenaar 1995). While TIM-1 system is usually utilized for the digestion of a wide range of food products, compound release, and absorption situation of nutrients in (Havenaar et al. 2013); TIM-2 has been designed for mostly fermentation and the changes in the microbiota in human proximal colon (Ramasamy et al., 2014). In addition, Verwei et al. (2016) developed a new simplified system using TIM-1 with some modifications. In that system, there is only one intestinal compartment and no ileal effluent like original system. TIM systems are also used for drug release studies under fasted and fed conditions in pharmaceutical studies (Verwei et al. 2016). TIM systems have many advantages comparing to the traditional in vitro systems such as allowing the control of temperature, pH and transit time, continuous enzyme secretion, evacuate the gastric and intestinal medium and ability for regulating transition of water and digested food (Al Hasawi et al., 2017; Teixeira et al., 2017). As the meal transfer through the different part of system, it is exposed to different conditions owing to secretion of digestive fluids and the release and absorption situation of water and nutrients present in meal. TIM systems have been increasingly drew attention of researchers. In this respect, researches performed by this method were presented year by year in Fig. 2.

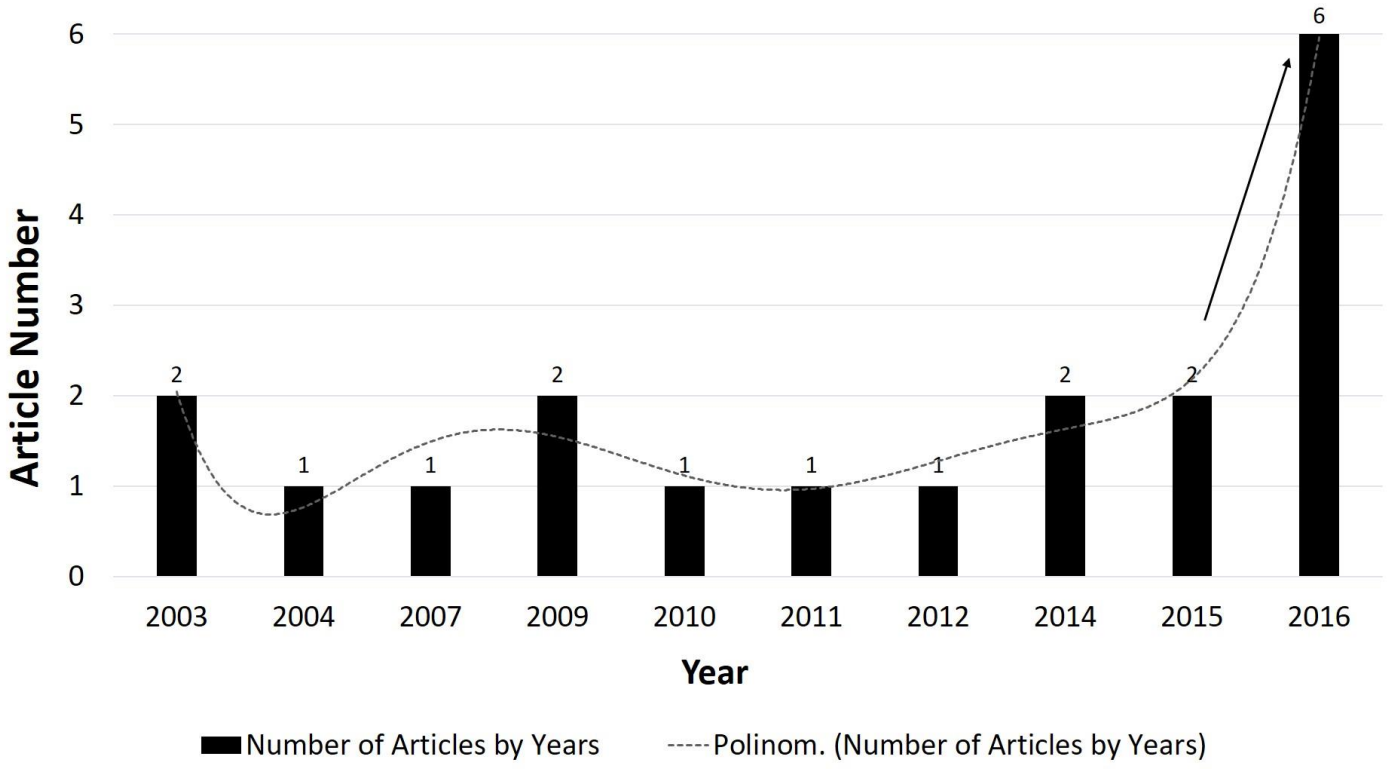

Fig. 2. Number of papers (in ScienceDirect, Taylor & Francis, ACS, RSC and Web of Science databases) published in the area of TNO gastrointestinal model in last 15 years.

2. TIM-1 Model System

TIM-1 model system consists of four parts; these are stomach, duodenum, jejunum and ileum connected to each other with peristaltic valve pumps (PVP) that are used for transfer of chime (Minekus, 2015). This system, imitate the peristaltic motions and gastrointestinal transit along with regulating the pH, bile salt and enzymatic activity via computer-controlled way (Brouwers et al. 2011). Advantages of the system can be summarized as changing pH gradually, secreting the enzymes continuously and absorption and removal of water and digested foods with a dialysis system, which allows measuring the bioaccessibility of matrixes (Minekus, Marteau and Havenaar 1995). This system has been used for the different type of food products such as dairy and wheat and fish proteins, as well as the evaluating the bioaccessibility of pharmaceutical drugs (Havenaar et al. 2016). As mentioned before, TIM consists of two main mechanical parts, TIM 1 and TIM 2, which are used for the monitoring absorption-ready compounds of foods or pharmaceuticals.

TIM-1, mimics the stomach and small intestine, separated into three parts (Fig. 1a). There are two dialysis systems (L), which allow removing the nutrients in both outer side of small intestine. At every stage of study, system allows user to take sample of material composition. When the food enters the stomach (A), gastric acid, lipase and pepsin, which are presented in section G, are pumped into the stomach and mixed with food immediately. To mimic the stomach movements, mix is shook by pushing forward and backward (between 2 compartments in section A). Low acidity of stomach inhibits the most of bacteria; proteins and lipids are exposed to the initial break down by enzymes pepsin and lipase, respectively and smaller peptides, fatty acids and glycerol are formed. After this step, food gradually leaves the stomach to the small intestine, which has three parts including duodenum (C), jejunum (D) and ileum (E). In Duodenum, first part of small intestine, bicarbonate is secreted from duodenum secretion part (H) to neutralize pH. To emulsify fat, bile is secreted and to digest carbohydrates, fats and proteins, pancreatic enzymes are secreted from the duodenum secretion section (H). In this step, enzymes break down the macromolecules (fat, carbohydrates and proteins) into smaller molecules, which are proper to absorb through to the gut wall and bloodstream that will be available in the later steps. As mentioned above, two dialysis system, ensure the digested materials removed, that attached to the jejunum and ileum. At the end of the TIM-1, non-digested or non-removed components pass through the TIM-2. Studies performed by TIM- 2 were represented in Fig. 3a.

Krul et al., (2000) visualized the presence of heterocyclic aromatic amines in the intestinal region by TIM. The effect of matrix affinity, absorption and the rate of passage through the digestion were assayed on 4 heterocyclic amines as model compounds for food mutants. The result is that TIM can be used to display mutagenic compounds such as anti-mutagenic compounds (Krul et al., 2000).

Larsson et al. (2016) investigated *in vitro* digestion of cod liver oil, which has an important role in the prevention of cardiovascular diseases. For this purpose, oxidation products, pre-emulsification of the oil and the addition of antioxidants have been studied for the oxidation of GI. Aldehydes were emerged in gastrointestinal tract. It has been observed that emulsification of fat is a vigorous lyotropic effect in the gastric phase but an inhibitory effect of oxidation in the intestinal phase (Larsson et al. 2016).

In another study, Ribnicky et al. (2014) compared TNO intestinal model (TIM-1) of the upper gastrointestinal tract with intestinal absorption / bioavailability of blueberry anthocyanins under different digestion conditions. When compared to the fasting state, it was observed that the biological availability of six

of the seven wild anthocyanins measured by a high-fat meal presence HPLC changed, but there was no significant effect on the amount of anthocyanin in ileal efflux. Biological accessibility of malvidin-3-glucoside in starvation and feeding was about 16%, while that of delphinidin-3-glucoside was 4.3% and 9.7% in starvation and feeding situations, respectively (Ribnicky et al. 2014).

Ferulic acid (FA) is the most important contributor to the in vitro antioxidant capacity of wheat seed. Many studies have yielded very variable results on ferulic acid bioavailability (0.4-98 %). Anson et al. (2009) aimed to monitor the release of FA during the gastrointestinal (GI) transition. It has been reported that wheat fractions and breads (<1%) show low bioavailability to FA. However, when free FA is added in flour (approximately 60%), bioaccessibility of FA is increased. In order to increase the bioavailability and systemic health effect, it was stated that new processing developments should be considered to increase the free FA in the grain matrix (Anson et al. 2009).

Uriot et al. (2016) aimed to determine the survival and adaptation capacity of 30 *S. thermophillus* strains from the yoghurt bacteria in the intestinal region in terms of urease, low temperature shock protein and amino acid decarboxylase enzymes. The survivability status of 4 strains was determined by TIM. Urease and heat shock have indicated that the predominant species of protein functions survive better. In addition, the bacterial cells of the LMD9 strain were transported in fermented milk, leading to a better development in fermented products than non-fermented products. In addition, recombinant *In Vivo* Expression Technology (RIVET), when applied to the LMD9 strain, showed that the hisS enzyme (producing tRNA) present in such species exhibited activity in the artificial gastric environment (Uriot et al. 2016).

Miszczycha et al. (2014), in another study, aimed to determine shiga toxin producing *E. coli* in contaminated raw cheeses by TIM. In the produced cheeses, *E. coli* growth was 2 log during initial production and 4 log during ripening period. The *E. coli* count decreased in artificial stomach environment and intestinal environment. Depending on the species, the amounts found in the intestinal and stomach environments have changed (Miszczycha et al. 2014).

3. TIM-2 Model System

Continuous dynamic models of the large intestine let more in-depth study of the effect of the gut microbiota on selected food molecules. TIM-2 (Fig. 1b) is designed for the dynamic model of the colon that is based on the same technology as TIM-1 (Minekus et al. 1999). It is well known in vitro GIT model, developed by Havenaar and Minekus (1996) in Netherlands. It has peristaltic movement and mixing forces. TIM-2 is constituted of glass jackets with stretchable inner wall where water is pumped into the space in between to maintain a constant temperature of 37°C. To reflecting in vivo conditions, TIM-2 medium contains nutrients, resistant starch, vitamins, minerals, bile and pancreatic enzymes. Studies performed by TIM- 2 were represented in Fig. 3b.

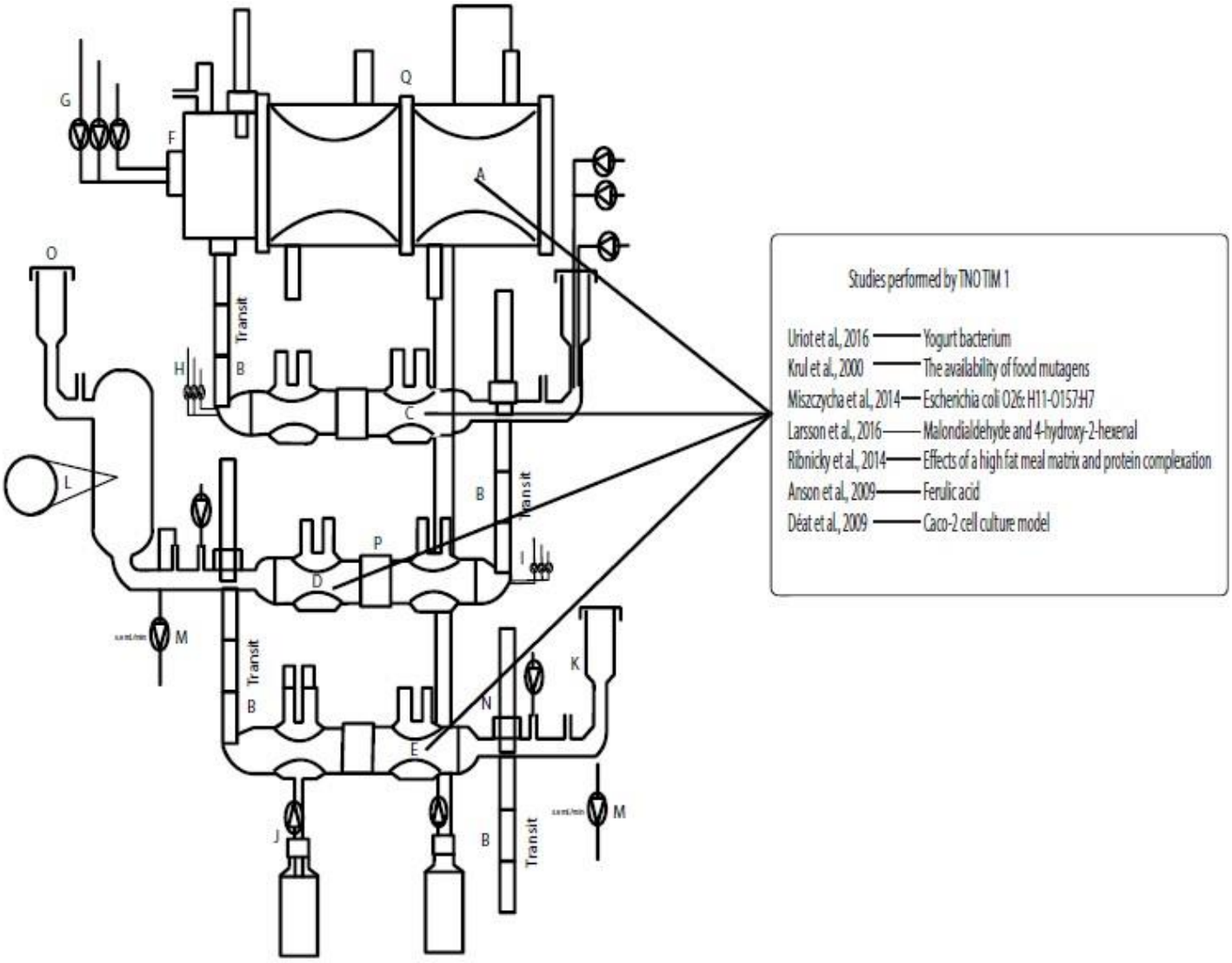

Fig. 3a. Studies performed by TNO TIM 1

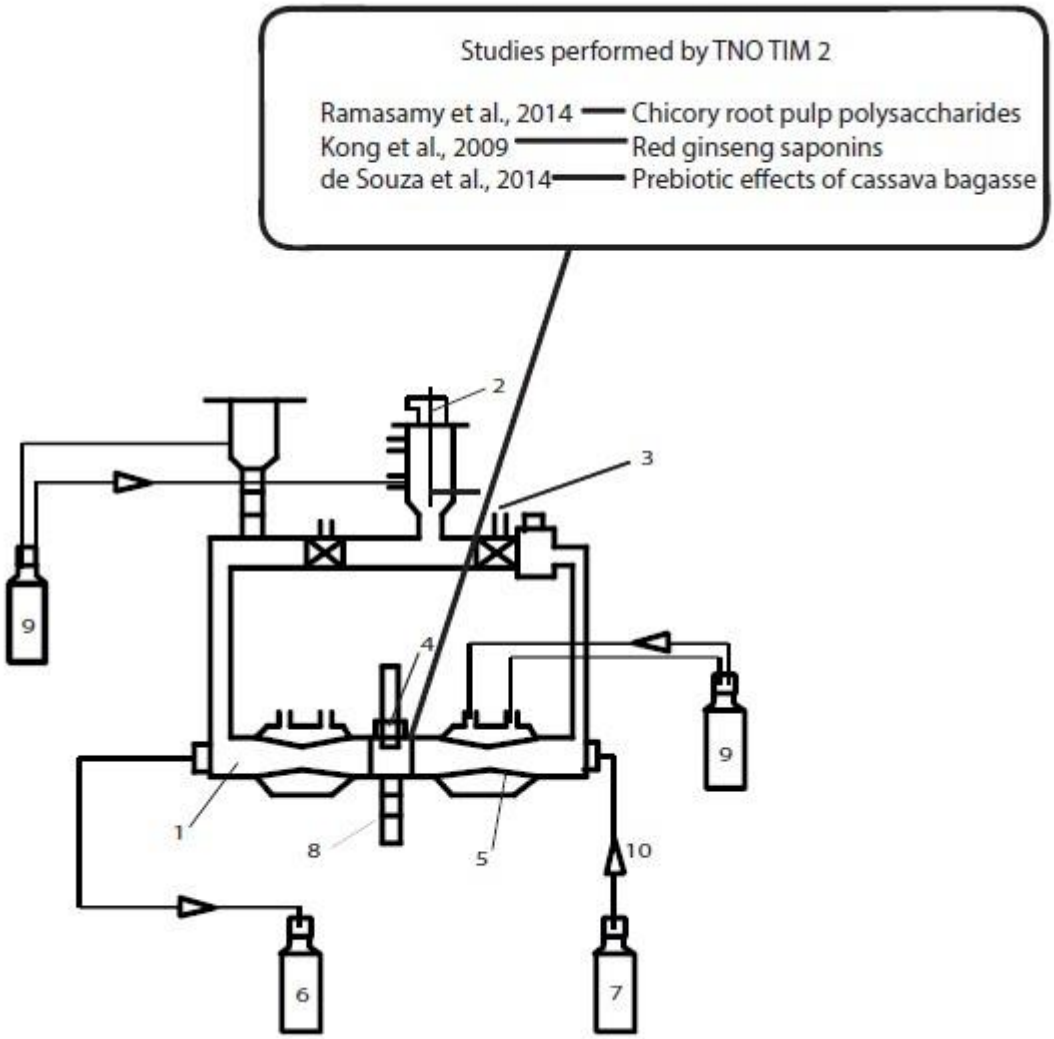

Fig. 3b. Studies performed by TNO TIM 2

TIM-2, which has many bacterial species, simulates the colon or the large intestine. This part allows measuring the interactions between microbiota and fibers. In TIM-2 bacterial species metabolize the food residuals. At every step of TIM-2, food components can be collected and analyzed. Using TIM- 2 how food or medicine affect the microbiota cane be monitored and also small molecules like short chain fatty acids, which are produced by gut microbiota can be measured.

De Souza et al. (2014) compared the prebiotic effect of cassava bagasse in obese and weak microbiota by TIM 2 instrument. Inulin and cassava have been reported to promote *Bifidobacterium* in lean microbiota. When cassava bagasse compared with standard, cassava bagasse stimulate *Bifidobacterium* 1738 fold than standard, inulin stimulate *Bifidobacterium* 169 fold than standard. As a result of fermentation of cassava bagasse, cassava bagasse produces more propionate and less butyrate than others prebiotics (inulin, standard). It is noted that the methodology used herein can be used as a rapid screening tool for the assessment of prebiotic activity of non-digestible substrates (de Souza et al. 2014).

In another study, Kong et al. (2009) determined *in vitro* metabolism of red ginseng in intestinal microbiota by TIM-2. It has been stated that large molecules such as polyphenols are not digested in the small intestine. In addition, the stability of ginsenosides in acidic media was demonstrated by HPLC-MS equipped with FTICR-MS. The results of this study have opened the way for better understanding of chemical and phytopharmaco kinetic parameters in bioavailability (Kong et al. 2009).

In a related study, the pathway observed in the intestinal fermentation in the TIM 2 model system of the pulp (obtained from untreated and processed, containing 4 times more soluble pectin than untreated) from the rhizome root was observed. Samples adhering to the human intestinal tract for 48 hours were fermented for 2 hours. Processed chicory samples were fermented less efficiently than the other samples. According to the results of the study, currently fermented fibers reduce the fermentation demand of microorganisms and reduce the fermentation rate of insoluble fibers. This shows that microorganisms prefer untreated fibers to those processed (Ramasamy et al. 2014).

4. Conclusion

Foods that consumed by humans expose many different conditions during digestion including break-up, release and absorption. To fully understand digestion of dietary components several methods have been conducted for years. *In vivo* and *in vitro* studies have been used for bioaccessibility and bioavailability of food components. However, both systems have their own disadvantages. Therefore computer controlled, multi-compartmental tool mimics the human GI system for adequate data of pathways followed by foods in fast and fasted conditions. In this review, it was outlined that TNO TIM model system in the

context of working principles and its parts. Findings displayed that bioaccessibility and bioavailability of functionally active components such as fatty acids, dietary fibers and polyphenols could be monitored. Also by TIM-2, behavior of gut microflora can be monitored in healthy conditions. In the future, this device might be used to gain insight into the bioaccessibility and bioavailability of functional foods. For example, it could be determined whether probiotic products sold in markets are actually probiotic foods. It may be developed with another part of human metabolism system such as liver and lung. This device could determine the metabolism of bioactive compounds and how the effect of bioactive compounds in target organ. This device can be designed for child, baby, young, middle age and aged human digestion system with set up enzyme quantity or speed of digestion.

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