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An *in-vitro* upper gut simulator for assessing continuous gas production: A proof-of-concept using milk digestion

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ABSTRACT

Using gases as biomarkers is receiving increasing attention across the field of gut health. The use of *in-vitro* digestion systems that measure gas is common to understand some of the complex systems that create these gases and previously have been conducted using long time period sampling schemes which miss important signatures regarding the dynamics of gas production. Here the development of a mono-compartment *in-vitro* digestion simulator capable of recording vital dynamic information in real-time including: CO_2 concentration; high sensitivity H_2 concentration; and pH are presented and validated utilising milk. The impact of a simplified bacterial model, bacterial population size and the presence of lactase are investigated. The favourable gas production outcomes are obtained when lactase is present, at 10^6 CFU of bacteria, in good agreement with clinical observations. This proof-of-concept system demonstrates reliable and repeatable results and has the potential to enhance the information capacity of current and future *in-vitro* simulators.

1. Introduction

Gas biomarkers are receiving increasing attention across the field of gut health (Ou et al., 2015). The gas profiles of the gut can potentially be used for diagnosis and monitoring of certain gut disorders including nutritional malabsorption, irritable bowel syndrome (IBS), and small intestinal bacterial growth (SIBO) to name a few (Kalantar-Zadeh et al., 2018).

Gas generation within the gut is the result of biochemical reactions and bacterial fermentation along the gastrointestinal tract. There are several tools for assessing these gases *in-situ* including swallowable gas sensing capsules (Ciuti, Menciassi, & Dario, 2011; Kalantar-Zadeh, Ha, Ou, & Berean, 2017), calorimetry (King, Elia, & Hunter, 1998), direct tubing (Levitt & Bond, 1970), breath tests (Romagnuolo, Schiller, & Bailey, 2002) as well as many imaging techniques (Murray et al., 2014; Perez, Accarino, Azpiroz, Quiroga, & Malagelada, 2007).

Alternatively, the gas production within the gut can also be modelled and assessed indirectly by *in-vitro* gut simulators. These simulators have shown to provide useful information about the gut digestive processes and by-products as a result of food digestion. Incorporating food absorption is a fundamentally complex task in any simulation (Gibney et al., 2005), but information pertaining to the endogenous chemical reactions and bacterial fermentation processes can be obtained using *in-vitro* systems under standardised conditions.

Digestion relies on chemical interactions and mechanical forces within the gastric phase. As the digesta moves though the gut and into the small bowel, bacterial fermentation becomes the dominant source of gas production. Fermentation constitutes a series of bacterial metabolic processes that break down and consume the food substrates.

It has been widely accepted that H_2, CH_4 and CO_2 are the most important functional gases of the gut that are found at relatively large concentrations (King & Toskes, 1979; Levitt, 1989; Moon, Li, Bang, & Han, 2016). The digestive activities of enzymes and chemical components throughout the gut, especially within the upper digestive tract, have significant impact on the onset of fermentation (Woolnough, Monro, Brennan, & Bird, 2008). While the gas production generated in the oral and gastric phases are dominated by chemical (mostly CO_2) and enzymatic interactions, gas production from bacterial activities begin when the digesta passes through to the jejunum and ileum segments of the small bowel. The overall fermentation of food intensifies when the digesta reaches the terminal ileum and large bowel.

Nowadays, *in-vitro* digestion simulators are popular tools for the exploration of specific digestive functions, due to cost effectiveness, repeatability and the ability to control environmental conditions (Chen

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et al., 2011; Guerra et al., 2012; Payne, Zihler, Chassard, & Lacroix, 2012). Such digestion simulations are represented by environmental parameters used for emulating the gut including chemical mix of digesta, enzymatic content, pH and anaerobic conditions, and bacterial models (Hur, Lim, Decker, & McClements, 2011; Minekus et al., 1999; Molly, Vande Woestyne, & Verstraete, 1993; Oomen et al., 2003; Payne et al., 2012). Some *in-vitro* systems are equipped with modules that measure the total volume of gases produced (Wang & Gibson, 1993) or measure headspace gas constituents that are sampled between 1 and 6 h increments (Moon et al., 2016). So far, all systems do not incorporate continuous gas measurement capabilities. For this critical reason, the current analysis by such systems misses important temporal gas production information relating to source, namely chemical, enzymatic, or bacterial fermentation (Coles, Moughan, & Darragh, 2005). A continuous gas monitoring platform overcomes this allowing to identify a specific source of gas production by observing temporal gas production information.

To advance current systems, we develop a mono-compartment digestion simulation of the oral, stomach and small intestine (SI) fitted with a gas measurement system that can record gas production (CO_2 and H_2) and pH in real-time. This paper shows a proof-of-concept study to validate this developed system using bovine milk (which will be referred to as milk) as the food substrate to investigate the short-term gas productions profiles during simulated milk digestion. Milk is chosen as it is a well-studied food and the cause of well documented gut disorders such as lactose intolerance. Symptoms of bloating and flatulence in lactose intolerance patients are commonly observed (Lomer, Parkes, & Sanderson, 2008; Matthews, Waud, Roberts, & Campbell, 2005; Schaafsma, 2008; Swallow, 2003; Vilotte, 2002).

The formulation of the digestive liquid, including the explored enzymatic content, used in the presented study is based on the work of Minekus et al. (2014). The digestion simulator investigates the short-term temporal information of gas production during the digestion of milk as it is rich in macronutrients (carbohydrates, proteins, and lipids). The *in-vitro* digestion simulator is also used for investigating the effect of a simplified bacterial model on gas production within the upper gut. Common bacterial communities seen in the SI are from the *Lactobacillaceae* and *Bacteroidaceae* families (Drasar, Shiner, & McLeod, 1969; Simren et al., 2013). A simplified bacterial model has been employed, containing bacteria from these families that are representative of the SI gut flora, specifically the strains *Lactobacillus acidophilus* NCFM and *Bifidobacterium lactis* Bi-07. These strains have been observed in human SI samples (Altermann et al., 2005; Ventura, Turroni, Lugli, & van Sinderen, 2014). These bacteria have been noted for the beneficial effect on the host and used in many clinical trials to help patients with gut disorders, reducing bloating severity (Floch, 2003; Ford et al., 2014; Rousseaux et al., 2007). To observe the effects that bacteria have on the milk digestion, simulations have been conducted with and without the bacterial model to investigate the impact on gas production within the simulated upper gut.

2. Experimental

2.1. Developed in-vitro digestion simulation system

The core of the simulator developed for this investigation is a mono-compartment (Fig. 1) *in-vitro* digestion unit based on the work by Oomen et al. (2003) utilising the upper digestion simulation protocol from Minekus et al. (2014) with an integrated sensor array and electronic units for real-time measurements. The system was designed to simulate the oral, gastric and SI aspects of digestion. A 250 ml Schott bottle with single septa port, and a custom made high density polyethylene (HDPE) housing was used. The HDPE housing contains the sensor array which can also accommodate a pH probe connection. A wireless RF pressure monitoring system (ANKOM, USA) was equipped to maintain a constant pressure within the digestion simulation unit and

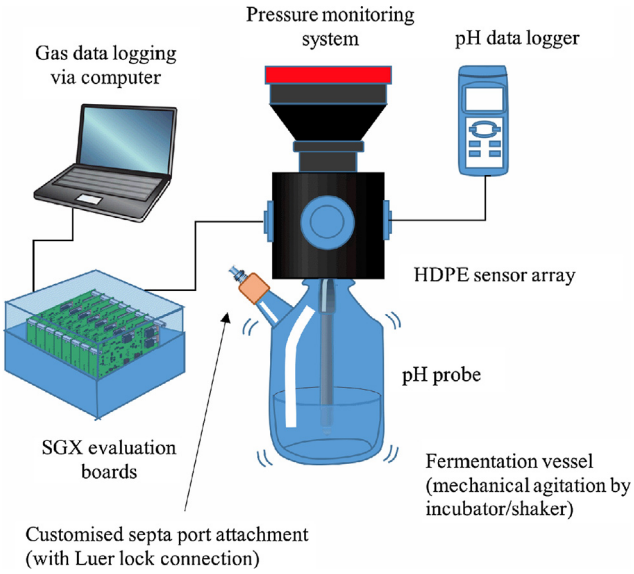

Fig. 1. Schematic of the mono-compartment *in-vitro* digestion simulation set up. Photo of the setup has been added to.

log internal pressure data. A RADTEK SWB20D incubator/shaker bath (Thermo Fisher Scientific, Australia) was used to agitate (0.5 rpm) and maintain the temperature (37 °C) during the simulations.

The gas sensors used were: a near-infrared sensor (IR25TT-R - SGX sensortech, Poland) for measuring CO_2, a thermal conductivity sensor (VQ546M - SGX sensortech, Poland) for measuring H_2 and an electrochemical O_2 sensor (EC410 - SGX sensortech, Poland). The O_2 sensor was used for monitoring the complete purging of the headspace within the digestion simulation units. These particular sensors were chosen based on the work of Ou et al. (2015). The sensors were connected to a computer *via* SGX Sensortech sensor evaluation boards (ECVQ-EK3/IR-EK2 - SGX sensortech, Poland) for data logging and analysis. The pH data was recorded onto a SD-card *via* a SD-230 Logging ORP/PH Meter (Rapid Technology, Australia).

2.2. Materials

All materials used in the *in-vitro* digestion simulation were of technical grade and purchased from Sigma Aldrich, excluding the milk and digestion enzymes. Amylase (38,000 U/mg), pepsin (10,000 U/mg), pancreatin (USP × 1), and lactase (10,000 U/mg) were purchased from Southern Biological (Australia). Milk was sourced from the local supermarket and stored at 4 °C. As also mentioned in the introduction, the bacteria used in the *in-vitro* digestion simulation were *Lactobacillus acidophilus* NCFM and *Bifidobacterium lactis* Bi-07, which were sourced in the form of a probiotic capsule (Inner Health Plus Diary Free, Ethical Nutrients). The microbiome size and diversity is highly dependent on many factors (Lozupone, Stombaugh, Gordon, Jansson, & Knight, 2012) but these particular bacteria, of the phylum *Firmicute*, have been found at relatively high concentrations in the gut and as such is a good starting point to form a simplified model (Kleerebezem & Vaughan, 2009) for investigating the impact on gas production during digestion simulations. The simplified bacterial model chosen for the experiments is not methanogenic and hence CH_4 was not observed in the measurements.

2.3. In-vitro simulation

The *in-vitro* simulation of the upper digestive tract used materials and design protocol as by Minekus et al. (2014) and consisted of an oral phase, gastric phase, and the small intestine phase. All simulated digestion fluids used in the simulations were made on the previous day