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THE ROLE OF SWEET POTATOES BUFFERING CAPACITY IN GASTRIC DIGESTION

BACHELOR'S IN FOOD SCIENCE AND TECHNOLOGY

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ABSTRACT

THE ROLE OF SWEET POTATOES BUFFERING CAPACITY IN GASTRIC DIGESTION

During gastric digestion, spatial and temporal pH gradients exist in the meal, which may impact food nutrient hydrolysis due to pH-mediated enzyme activity. These pH gradients are influenced by both the type of meal as well as the meal buffering capacity. As such, it is important to understand the relationship between the initial food matrix, its buffering capacity, and the resulting pH gradients that occur during gastric digestion. This study will utilize a mechanical gastric model, the Human Gastric Simulator to study the pH gradients found during gastric digestion of sweet potato snacks. Sweet potato snacks will be processed using 2 methods: frying and blanching-frying. After processing, the food buffering capacity will be measured. During gastric digestion, samples will be taken to understand the pH distribution and the regional properties of the gastric digesta after each meal. The results show that regional pH distribution varies, and it is influenced by buffering capacity. Moisture analysis show a greater trend for all treatments and particle size distribution analysis show what is logical, smaller and more particles at the end of the digestion.

Key words: buffering capacity, enzyme activity, food digestion, gastric digestion, mixing

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RESUMEN

EL PAPEL DE LA CAPACIDAD TAMPÓN DE LAS PATATAS DULCES O BONIATOS EN LA DIGESTIÓN GÁSTRICA

Durante la digestión gástrica, existen gradientes espaciales y temporales de pH en la comida, hecho que puede afectar a la hidrólisis de los nutrientes de los alimentos debido a la actividad de la enzima mediada por el pH. Estos gradientes de pH están influenciados tanto por el tipo de comida como por la capacidad tampón de esta. Como tal, es importante comprender la relación entre la matriz alimentaria inicial, su capacidad tampón y los gradientes de pH resultantes que se producen durante la digestión gástrica. Este estudio utilizará un modelo gástrico mecánico, el simulador gástrico humano, para estudiar los gradientes de pH encontrados durante la digestión gástrica de los boniatos. Los boniatos serán procesados usando 2 métodos: fritura y escaldado – fritura. Después de triturar y procesar la comida, se medirá la capacidad tampón del alimento. Durante la digestión gástrica, se tomarán muestras para conocer la distribución del pH y las características regionales en el estómago después de cada comida. Los resultados muestran que la distribución regional del pH varía, y está influenciada por la capacidad tampón. El análisis de humedad muestra una buena tendencia para todos los tratamientos y el análisis de distribución de tamaño de partículas muestra lo que es lógico, partículas más pequeñas y más pequeñas al final de la digestión.

Palabras clave: capacidad tampón, actividad enzimática, digestión de alimentos, digestión gástrica, mezcla

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1. INTRODUCTION

1.1 SWEET POTATOES CHARACTERISTICS AND PROPERTIES

Orange fleshed sweet potatoes (*Ipomoea batatas*) grow properly in tropical, subtropical and temperate areas. Their distribution is now worldwide but its origin was in the New World and they were introduced into Spain, India and the Philippines by Spanish explorers in the 15th and 16th centuries. In parts of Africa, Asia and the Pacific, sweet potatoes are an important staple crop (Woolfe, 1992; Bovell-Benjamin, 2007).

Sweet potatoes are considered as a good source of natural health-promoting compounds because they are nutritious tubers, low in protein and fat but rich in carbohydrates and dietary fiber, vitamins, minerals, antioxidants such as β-carotene (which is the primary vitamin A (VA) forming carotenoid) and anthocyanin (Bovell-Benjamin, 2007; Bengtsson et al., 2008; Wu et al., 2008; Teow et al., 2007; Burri, 2011). They also have antioxidative, antiinflammatory, antitumor, antidiabetic, antimicrobial, antiobesity, antiaging effects (Wang et al., 2016).

The valuable nutritional qualities of sweet potatoes have resulted in their selection as one of the foods tested for long-term space travel (Wilson and others 1998). In addition, there are segments of the global population at risk for VA deficiency. There are 190 million preschool children and 19,1 million pregnant women from low-income and food-deficit countries (Burri, 2011). According to a previous report (2011), the current world production of sweet potatoes is 106.5 million metric tons, which is much higher quantity than the 2.08 to 11.68 million metric tons that would be required to supply 100% of the VA for the people most at risk for VA deficiency in the world, suggesting significant opportunities for increasing global sweet potato consumption.

There are a great variety of cooking and processing methods for sweet potatoes (Woolfe, 1992; Emenhiser et al., 1999; Sulaiman, et al., 2003; Rodriguez-Amaya & Kimura, 2004; Low & van Jaarsveld, 2008). They can be cooked many different ways prior to consumption, cooking methods include boiled, steamed, roasted, deep fried, baked and microwaved (Bengtsson et al., 2008; Burri, 2011).

1.2 GASTRIC DIGESTION PROCESS

1.2.1 Digestion of foods

The release and absorption of energy and nutrients from foods takes place during the digestion process. It is vital to understand how food interacts and reacts to gastric fluids for studying food breakdown during this process (Bornhorst, 2017). Physical and chemical forces act in combination to break down ingested food into small molecules in the digestive tract. During digestion, food particles became smaller to facilitate the

incorporation of nutrients into the bloodstream. There are some organs which play a key role during this process: mouth, stomach and small intestine. Food is disintegrated into small size in the mouth and stomach and the main nutrient absorption takes place in the small intestine (F. Kong & Singh, 2008).

The first stage of food digestion is the oral cavity, where food is ingested, chewed and broken down physically by mastication and enzymatically by mixing it with saliva forming a bolus (Van Der Bilt et al., 2006; Kong & Singh, 2008). The majority of the remaining food breakdown occurs in the gastric environment after mastication. When bolus particles are reduced to a certain size, they can be swallowed and transported through the esophagus to the stomach by the mechanism of peristalsis (F. Kong & Singh, 2008). Peristalsis is an advancing wave of contraction of the walls of a flexible conduit. This movement forces the contents forward throughout the gastrointestinal tract (Siddiqui et al., 1991; Bornhorst & Singh, 2012). Physical and chemical breakdown continue when the bolus reaches the stomach as a result of peristaltic contractions and gastric secretions. After that, food goes through the small and large intestines where the chyme or digested food is mixed to facilitate absorption of nutrients and fermentation. The digestion process ends at the anus (Bornhorst & Singh, 2014).

The stomach is formed by the cardia, fundus, and antrum. The cardia is filled with mucin-secreting cells. In the fundus, HCl is secreted by parietal cells and chief cells secrete pepsinogen which is converted in to pepsin and lipase. The antrum is filled with mucus-secreting cells (Bornhorst & Singh, 2014). The stomach is divided into two major regions: the proximal stomach or upper part and the distal stomach, or lower part. The proximal part is comprised of the fundus and body, and acts as a reservoir. The food bolus remains in this compartment until it moves forward into the distal region. The distal stomach is comprised of the antrum acts as a tank, mixer, grinder and sieve (Meyer, 1980) in a complex feedback control system that simultaneously breaks down ingested foods while mixing them with gastric acid and digestive enzymes. There is where most of the physical breakdown takes place due to the peristaltic contractions of the stomach walls (Kelly, 1980; Barret 2005; Kong & Singh, 2008). Besides the physical breakdown, chemical breakdown occurs because of gastric secretions. Gastric acid helps to soften food particle texture and pepsin and lipase (digestive enzymes) begin hydrolysis of nutrients (Bornhorst & Singh, 2014). The pylorus contracts to slow gastric emptying and results in further mixing of gastric contents. In the meantime, the stomach mixes water, fat and solid contents and the outcome of the mixing is called chyme. Gastric emptying is promoted by the more intense peristaltic waves, which allows gastric contents (fluid mixed with small particles mainly) to pass through the pylorus and enter the duodenum. The particle size of the food emptied through the pylorus is less than 1 to 2 mm during the fed state (Thomas 2006). Gastric emptying is the process in which food and drug materials that were broken down during gastric digestion are going to be emptied from the stomach (Bornhorst, 2017). The pylorus opens at blinking intervals to permit egress of small particles from the stomach (Meyer, 1980).

During the intestinal digestion is where most of the nutrient absorption and the remaining enzymatic breakdown take place because the food emptied from the stomach is mixed with the juices from the pancreas, liver and intestine (Kong & Singh, 2008; Bornhorst & Singh, 2014). The small intestinal secretions contribute to the enzymatic hydrolysis of nutrients into small molecules that are able to be absorbed through the intestinal epithelium and carried into the bloodstream. It is well-known that the small intestine is divided into three parts: the duodenum, which secretes bicarbonate and helps to maintain pH digestion levels; the jejunum, where the majority of nutrients are absorbed; and the ileum, which is the only location where vitamin B12 and bile salts can be absorbed (Seidel & Long, 2006). The large intestine is the last point of the digestion process. Any material transferred to the large intestine is fermented by anaerobic bacteria. During this stage, only water and fermentation by-products are absorbed (Seidel & Long, 2006).

Food-related factors such as meal composition, structure, physical properties, buffering capacity and breakdown rate in addition to psychological processes such as gastric secretions, gastric motility and gastric emptying play an important part controlling the gastric digestion process (Bornhorst & Singh, 2014). The speed of the digestion process depends on the interaction of food properties with physiological events occurring within the gastrointestinal tract (F. Kong & Singh, 2008).

1.2.2. Physiological processes

1.2.2.1 Gastric secretions

The stomach is separated intro three regions: the cardia, the fundus/body or proximal stomach and the antrum or distal stomach (Bornhorst, 2017). Gastric secretions are made up of acid (HCl), enzymes (pepsinogen), mucus, bicarbonate and intrinsic factor (Barret 2005).

The key components of gastric fluids are secreted by gastric glands. As Barret (2005), Bornhorst (2017) and Heuman et al., (1997) studies postulate, the proximal stomach is fitted with the 75% of the gastric gland; hence, this is where most of the gastric secretions of acid, mucus, intrinsic factor, somatostatin, histamine and pepsinogen occur. Conversely, the distal stomach contains cells that secrete mucus, gastrin and somatostatin by G and D cells respectively and they act to regulate gastric secretions in the fundus and body.

The stomach secretes 2-3 L per day of 0.16 N HCl (Hersey &. Sachs, 1995; Bornhorst & Singh, 2014). The pH of the fasted gastric environment is between 1.4 and 2.0 so the basal acid secretion rate between meals is on average 1mL/min to maintain this low pH. Conversely, after the ingestion of food the gastric acid secretions may increase to 6 mL/min (Malagelada, et al., 1976; Dressman at al., 1990; Barret 2005).

Mucous and parietal cells are located at the top of the gastric glands. Mucous cells secrete mucus as well as bicarbonate ions. They are located in the superficial compartment of the stomach and they are secreted by the surface epithelial cells (Heuman et al., 1997; Bornhorst, 2017). Mucus is composed of glycoproteins, surface phospholipids, and water. It is a viscoelastic fluid which limits diffusion of acid and also takes care the inner stomach cells and musculature from its own acid secretions avoiding damages. Bicarbonate ions are secreted to protect the gastric mucosa from acidic secretions (Barret, 2005). Besides that, parietal cells, primarily simulated by histamine, acetylcholine and gastrin (Low, 1990, Hersey & Sachs, 1995), have a triangular form which contain H+-K+-ATPase. This mechanism acts as a proton pump to exchange K+ for H+ resulting in acid (HCl) secretion. Parietal cells secrete HCl, the main gastric secretory product of interest, and its secretion is modulated by food movement through the gastrointestinal tract (Barret 2014). They also secrete intrinsic factor, a protein that links to vitamin B12 and allows for its absorption in the ileum (Heuman et al., 1997).

Chief cells are located at the bottom of the gastric glands. These cells secrete the inactive precursor pepsinogen that is the inactivated form of pepsin. Pepsinogen is transformed into an active proteolytic enzyme, called pepsin, after coming in contact with acid by the removal of nine amino acids (Seidel & Long, 2006; Barret 2014). Pepsin has its maximum activity when pH < 2 and it is inactivated at pH > 5 (Kondjoyan et al., 2015). Chief cells also secrete gastric lipase, an important element in lipid digestion, responsible for 10 -30% of dietary triglyceride hydrolysis (Hamosh 1990, Gallier & Singh, 2012). Lipase has its maxim activity at pH 5 - 6 and its activity starts decreasing with pH < 4 (Gargouri, 1989).

The specific mediation of gastric secretory response depends on physiological and food-related factors once a meal is consumed (Bornhorst & Singh, 2014). These factors that may influence gastric secretion rate include: age (Feldman et al., 1996), smoking habits (Feldman et al., 1996), diseases (Schubert & Peura, 2008), ingestion and amount of food (Hersey & Sachs, 1995), intragastric pH (Calbet & Holst, 2004), meal viscosity (Marciani et al., 2001) and food buffering capacity (Fordtran & Walsh, 1973). Gastric secretions are useful to help with nutrients absorption, sterilize food from microbes and contribute to acid – enzymatic hydrolysis of the food, fact that is crucial for food breakdown (Barret, 2005).

1.2.2.2 Gastric motility

The movement of the stomach walls is known as gastric motility and is vital for gastric digestion (Bornhorst & Singh, 2014). Gastric motility patterns after consumption of a meal vary in the proximal and distal stomach regions (Bornhorst, 2017). The proximal stomach generates sustained muscle contractions, or tonic contractions of low frequency and amplitude (Lammers et al., 2009). In contrast, the distal stomach experiences phasic, peristaltic muscular contractions or antral

contraction waves (Barret, 2005). Antral contraction wave frequency, duration and intensity will impact the physical food breakdown and mixing in the stomach because they will change the forces, pressures, and flow profiles exerted on food particles (Bornhorst & Singh, 2014).

The stomach also plays another role in its function as a food reservoir known as receptive relaxation. What this term means is that the stomach has the ability to expand its volume after a meal. This allows the stomach to keep some of the meal in the proximal region before it moves to the distal region for further breakdown (Barret 2014; Bornhorst, 2017).

1.2.3. Gastric mixing process

The process of mixing is when one substance combines with another to eventually obtain an homogeneous substance (Cullen & Wiley, 2009; Rielly et al., 1994). Gastric mixing is facilitated during digestion through the peristaltic contractions of the muscular walls of the stomach (Bornhorst, 2017). Gastric mixing is nonhomogeneous and plays an important part during the processes of gastric digestion, including the rate of breakdown, pH distribution and gastric emptying (Guyton & Hall, 2006; Bornhorst & Singh, 2014).

As Bornhorst (2017) expresses, it has been demonstrated that there are several types of mixing in food and digestion systems: solid-solid mixing, solid-liquid mixing and liquid-liquid mixing. Solid-solid or mixing of meal components with each other, in external forces from the mixer such as phasic contractions from the gastric antrum constitute the driving force; solid-liquid or mixing of gastric secretions with a meal of solid food particles and liquid-liquid or the mixing of gastric secretions with a liquid meal, in which both external forces and diffusion influence mixing processes.

The pH distribution varies depending on the region of the stomach according to mixing process. The pH decreases to 2 because of the inactivation of salivary α-amylase (optimum pH 6 to 7) and simultaneous activation of gastric enzymes, such as pepsin (optimum pH 2 to 4). Gastric pH is regulated through a complex feedback-control system, similar to how many industrial processes are regulated (McCabe et al., 2005).

1.3 MODELS OF GASTRIC DIGESTION

There are both in vivo and in vitro models used to study the human digestion process. In vivo digestion studies can be performed using animal or human models. With these studies, the most important thing is to take in account the main objective and focus what has to be analyzed. For instance, there are tests to evaluate and measure gastric emptying such as scintigraphy (Seok, 2011), stable isotope breath tests, wireless

pressure and pH capsules, and magnetic resonance imaging (MRI) (Szarka & Camilleri, 2009). In addition, to measure the rate of food digestion and absorption, blood glucose concentration has been used to calculate the glycemic index (Bornhorst & Singh, 2014).

In vitro digestion models may include oral, gastric, small intestinal, and large intestinal phases. This type of digestion models has some advantages compared to in vivo studies such as greater repeatability with a larger number of samples, the ability to generate results in shorter time without ethical restrictions, less use of human resources, less expensive and being able to isolate specific parameters to study, among others (Minekus et al., 2014a). Two types of in vitro digestion models are used, static and dynamic models. Static models simulate chemical breakdown with a controlled temperature and speed (Wickham et al., 2009) but don't take into account physical breakdown. They are commonly done in a shaking water bath. In static models, the pH, the digestion time, digestive enzymes, salts, and temperature need to be considered (Minekus et al., 2014a). Conversely, dynamic model simulate chemical and physical factors and they are able to replicate peristaltic muscular contractions (Bornhorst & Singh, 2012). Dynamic models can control and regulate gastric emptying rate an gastric secretions and they simulate peristaltic contractions (Kong & Singh, 2010; Minekus et al., 2014a)

1.4 pH INFLUENCE ON FOOD BREAKDOWN

As several studies have observed, physical breakdown occurs due to mastication and peristaltic contractions and takes place primarily during oral and gastric digestion (Kong & Singh, 2010; Bornhorst & Singh, 2012) but there also exist chemical breakdown. The process of mixing of the gastrointestinal fluids into the food matrix is due to chemical breakdown. The molecules are available for absorption because there are chemical mechanisms, acid and enzymatic hydrolysis, that break food matrices intro nutrient molecules (Parada & Aguilera, 2007; Bornhorst et al., 2015).

It is commonly known that pepsin's optimal activity is at pH 2.0 (Kelly, 1980; Kondjoyan et al., 2015) and the inactivation of salivary α-amylase is at a low pH (2.0 approximately) (Dona et al., 2010). The pH in the gastric atmosphere may vary depending on the amount, type and buffering capacity of the meal. This fact could impact pepsin activity and therefore, protein hydrolysis. Nevertheless, part of the food ingested remains at high pH for longer period of time causing the α-amylase to stay active and hydrolyzing carbohydrates during gastric digestion (Bornhorst et al., 2014). This may result in faster diffusion of gastric acid through the bolus. As a result, the intragastric pH and food buffering capacity play a significant role in several food digestion mechanisms (Mennah-Govela et al., 2015).

1.5 BUFFERING CAPACITY

The resistance of a solution to change pH with the addition of an acid or alkali is known as buffering capacity (Van Slyke, 1992). Buffering capacity of a food is influenced by proteins and acid/base groups, such as salts and organic acids (Salaün et al., 2005; Puolanne & Kivikari, 2000).

Common methods that are used to measure buffering capacity include the addition of acid only, the addition of acid followed by alkali, the addition of alkali only or the addition of alkali followed by acid (Salaün et al., 2005). The current project will focus on acid buffering capacity to make the concept simple. It is really difficult to compare buffering capacity values between methods because each one is different and also the results vary. Previous authors have shown some examples of variations in methods such as sample preparation (i.e. sample diluted in water), pH endpoint, and quantity and concentration of acid or base added. Hence, is necessary to develop a standardized protocol to characterize foods based on their buffering capacity in the context of digestion to be able to compare buffering capacity values between different types of food (Mennah-Govela, 2017).

As Williams et al. (1968) and Calbet & Holst (2014) present in their studies, even though the influence of buffering capacity and gastric secretions with physical and chemical breakdown during gastric digestion is not currently known, there are evidences which show that both buffering capacity and gastric secretions are associated. According Gardner et al. (2002), it has been demonstrated that by knowing the amount of acid and the time needed to decrease the pH, (e.g. food buffering capacity) it is possible to calculate the amount of gastric juice secreted and the breakdown rate, both of which are related to gastric emptying.

2. OBJECTIVES

2.1 GENERAL GOAL

The overall objective of this project is to understand the relationship between the initial food matrix, its buffering capacity and the resulting pH distribution in the stomach during gastric digestion using a dynamic in vitro gastric model.

2.2 SPECIFIC GOALS

With the purpose of achieving the general goal, some specific goals have been determined:

- Study the pH gradients found during gastric (one and three hours) digestion of sweet potato snacks.
- Measure the buffering capacity of four samples: initial, with saliva, digesta top and digesta bottom.
- Analyze associated parameters from the proximal and distal stomach regions after 1 and 3 hours of gastric digestion, including moisture content and particle size distribution.

3. MATERIALS AND METHODS

3.1 SWEET POTATO COOKING PROCEDURE

Four different snacks were designed from the raw orange-fleshed sweet potatoes (*Ipomoea batatas*): fried, baked, blanched-fried, and blanched-baked. The current project is focused on the following cooking methods: fried and blanched-fried.

The sweet potatoes were cleaned with water to remove the dust remaining on the skin. They were cut in slices by using a cutting machine (Chef's Choice, Premium Electric Food Slicer, Model: 615) *(Fig.1)* to get sweet potato 3 mm slices thick.

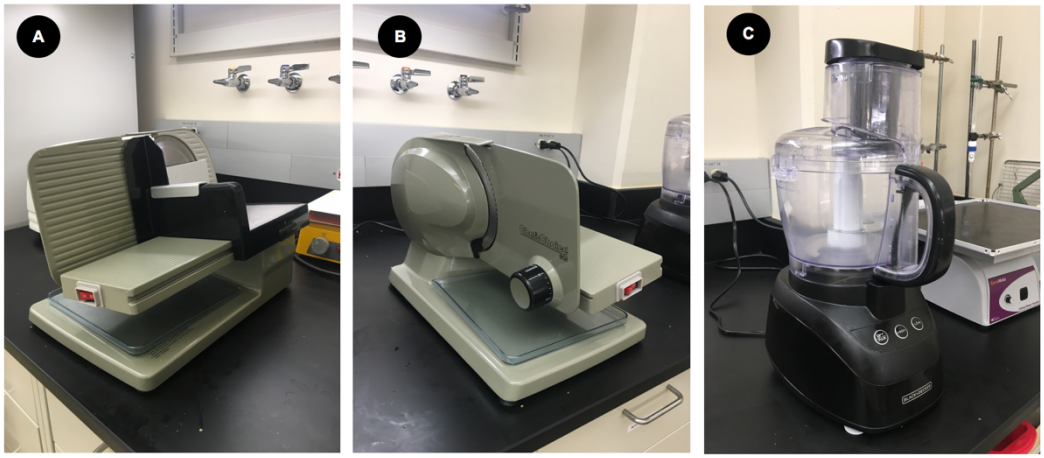

Figure 1. Cutting machine used to cut sweet potato slices (A, B); food processor (C).

The total cooking time for each method was selected based on preliminary trials to ensure similar results after cooking. For both methods, three batches of 300g (each batch) of raw sweet potato were cooked to ensure comparable sample heating conditions for each experiment. About 300g were needed for each batch of cooking for obtaining 100g approximately of cooked potatoes. For each digestion trial, 200g of processed sweet potatoes is needed.

Fried Snacks Protocol

For fried sweet potatoes, slices were fried in soybean oil (Kirkland Signature, Stratas Foods ™, distributed by Costco Wholesale corporation) at 134ºC for 5.5 min in a deep fryer (Hamilton Beach, Model: 35034, Type: DF11), and cooled for 20 min.

Blanched-Fried Snacks Protocol

One liter of water was put in a pot and warmed to 85ºC. Once the water achieved the temperature, 150g of sweet potatoes were immersed in water in the pot. They were

cooked for 1 min. Immediately after blanching, they were placed in a bowl with cold water for 1 min. They were placed on a tray with paper towels for 10 min before frying. The frying protocol was the same as described above. In blanched-fried cooking method, a thermometer was used to know the temperature of the water every time to ensure similar cooking conditions for each batch.

Figure 2. Raw sweet potatoes (A); sweet potatoes in frying process (B); the assembly of blanching (C).

3.2 SIMULATED DIGESTION

3.2.1 Simulated saliva formulation

All components to make saliva formulation were mixed in deionized water with pH of 7. The pH was adjusted with 0.01 N NaOH (Bornhorst & Singh, 2013). The necessary ingredients and quantities were: 1 g/L mucin (Sigma- Aldrich, MO, U.S.A.), 1.18 g/L a-amylase (from Bacillus subtilis, MP Biomedicals, Catalog Number 100447, activity of 160,000 BAU/g, Santa Ana, CA, U.S.A.), 0.117 g/L NaCl (Avantor Performance Materials, PA, U.S.A.), 0.149 g/L KCl (Fisher Science Education, IL, U.S.A.) and 2.1 g/L $NaHCO_3$ (Fisher Science Education, IL, U.S.A.) (Kong & Singh, 2010).

3.2.2 Gastric juice and lipase formulation

Simulated gastric juice was prepared by mixing some components in deionized water with pH of 1.8. The pH was adjusted to 1.8 using 0.1 N HCl (Bornhorst & Singh, 2013). The necessary ingredients and quantities were: 1.75 g/L mucin (Sigma-Aldrich, MO, U.S.A.), 10.24 g/L NaCl (Avantor Performance Materials, PA, U.S.A.) and 1 g/L pepsin from porcine pancreas (Sigma-Aldrich MO, U.S.A.). Lipase formulation was prepared by mixing 23.3 g/L of lipase in deionized water with pH of 4.5. The pH was adjusted to 4.5 using 0.1 N HCl (Bornhorst & Singh, 2013).

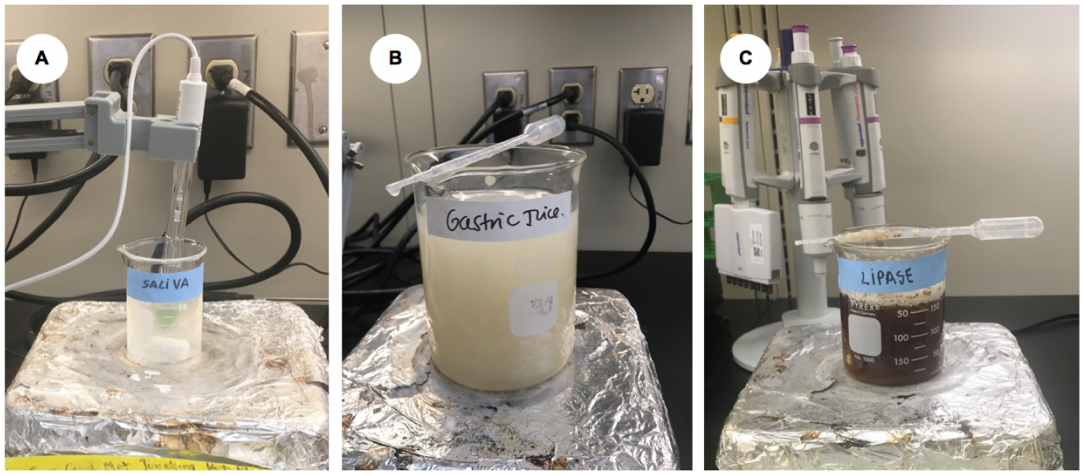

Figure 3. Saliva (A), gastric juice (B) and lipase (C) dissolutions in hot plates prepared for being used to during in vitro digestion.

3.2.3 Oral and gastric digestion conditions

Physical breakdown during oral digestion was performed using a food processor (Food Processor, Model: FP2500B, Spectrum Brands, Inc.). Physical breakdown during gastric digestion was performed using a dynamic in vitro gastric model, the Human Gastric Simulator (HGS) (Kong & Singh, 2010). Approximately 300 g coked sweet potato slices were processed in the food processor (Food Processor, Model: FP2500B, Spectrum Brands, Inc.) for 45 seconds at high frequency *(Fig 1)*. The HGS temperature was equilibrated at 37ºC (similar to human body), so a heating lamp and a dryer helped to achieve and maintain the temperature. 70 mL of gastric juice was placed into the stomach bag to represent the amount of gastric juice in fasting conditions. During the gastric digestion, a peristaltic pump (New Era 9000 Series Basic "Learn & Repeat ™", Pump Systems Inc.) was used to secrete 3 mL/min of gastric juice into the stomach bag and, simultaneously, syringe pump (Model: NE-300 "Just Infusion", Pump Systems Inc.) secreted 0.5 mL/min of lipase. The quantity of masticated sweet potatoes needed for gastric digestion varied between treatments to ensure equivalent dry matter content of

each meal, as they did not have the same initial moisture content (4% fried; 8% blanched fried). For fried digestions, 208 g of ground potatoes were used for each digestion and for blanched-fried digestions, 216 g were used for each digestion. After grinding in the food processor, saliva was mixed with the ground sample (41,6 mL of saliva for fried; 43,2 mL of saliva for blanched-fried), and this was placed into the HGS. When all was set up, gastric digestion could start.

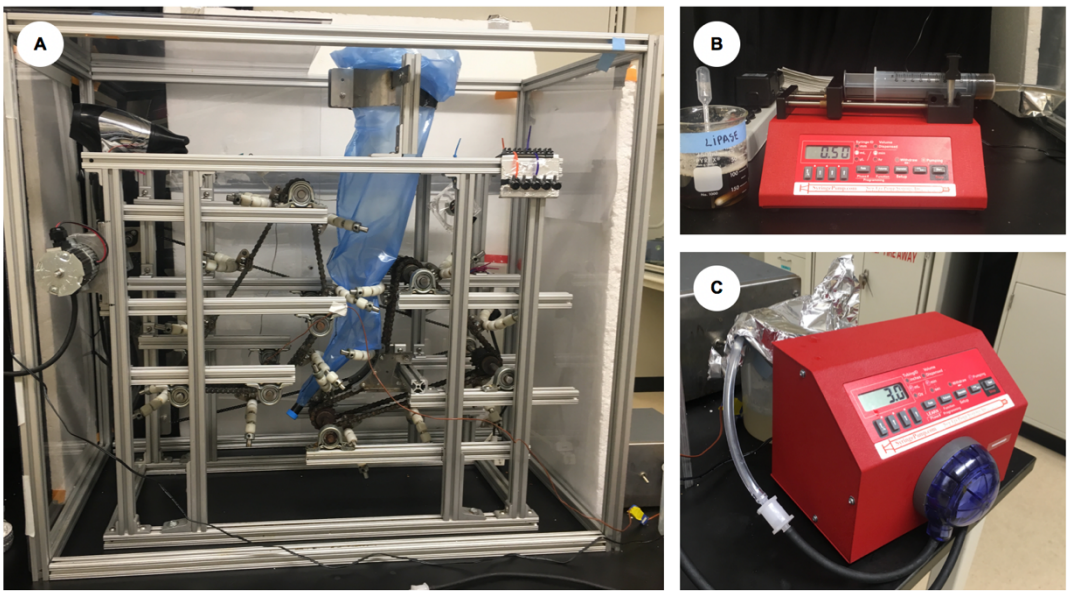

Figure 4. Human Gastric Simulator (HGS) (A); syringe pump (B) and peristaltic pump (C).

Digestions of 1-hour (timepoint samples: 0 min, 0.5 min, 30 min, top bag, bottom bag) and 3-hours (timepoint samples: 0 min, 0.5 min, 30 min, 60 min, 90 min, 120 min, 150 min, top bag, bottom bag) were performed. During the first 30 min of digestion, initial (0 min) and saliva (0,5 min) moisture samples were measured. Each 30 min a sample of 100g approximately was taken to analyze for moisture, particle size, and fat content. At the end of digestion, the stomach bag (Animal Reproduction Systems, ARSsales) was taken out from the HGS equipment with the purpose of measure inner pH distribution with the pH-meter (Fisher Scientific, Accumet AE150). Buffering capacity analysis of initial and saliva samples were performed during simulated gastric digestion experiments. After of measuring pH of the stomach bag, when the digestion was finished, buffering capacity analysis of digesta top and also digesta bottom were done. Simulated digestions were performed 12 times (i.e. 6 for each cooking method, three of 1 hour and three of 3 hours in each method).

3.3 SWEET POTATO BEHAVIOR DURING SIMULATED GASTRIC DIGESTION

3.3.1 Regional pH distribution

Once simulated gastric digestion was finished, pH was measured using a pH-meter (Fisher Scientific, Accumet AE150) in different key points of the stomach bag: at the top (red line) and in the middle (green line) of the proximal stomach in three spots (left, middle and right); at the top, in the middle and in the emptying from left to the right as well in the distal stomach.

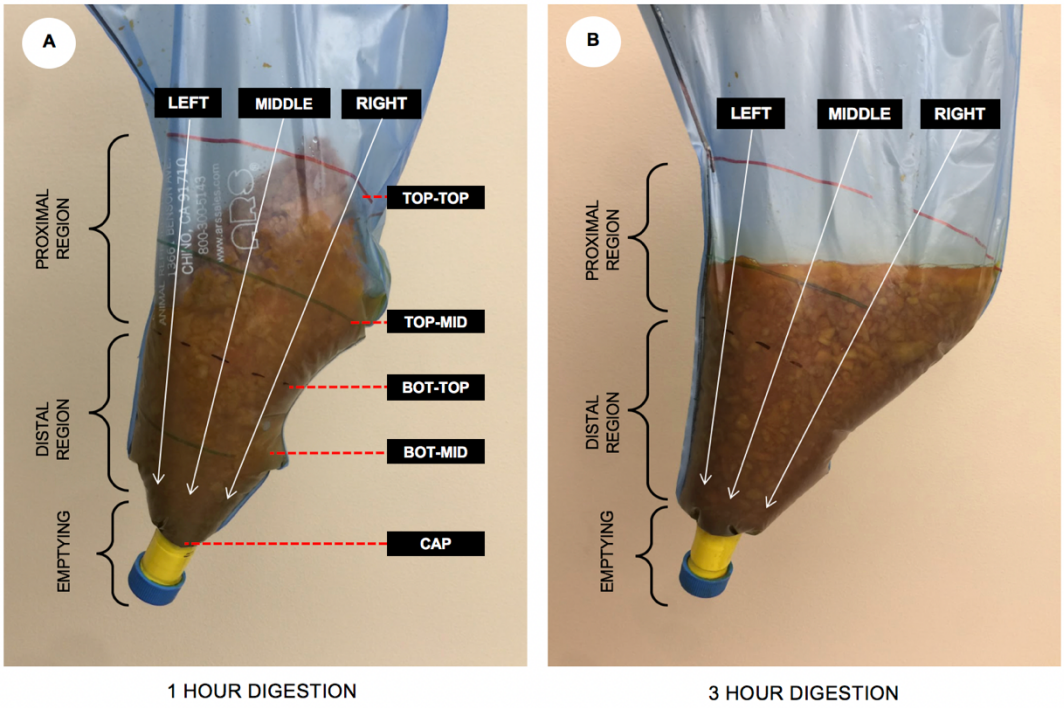

Figure 5. Stomach bags of blanched-fried method of 1 (A) and 3 hours (B) after the simulated digestion in the HGS.

3.3.2 Moisture content

Moisture was determined gravimetrically by drying in a vacuum oven (Thermo Fisher Scientific, Lindberg/Blue M Vacuum Ovens VO914/ VO1218 /1824) for 20h at 110ºC ~25inHg pressure, until constant weight. Three pans (A, B, C) per timepoint were labeled and introduced in the oven to pre-dry for 30 min. Their masses were recorded, and samples was put into each pan. The total mass was written again. After 20h, the pans were weighed again.

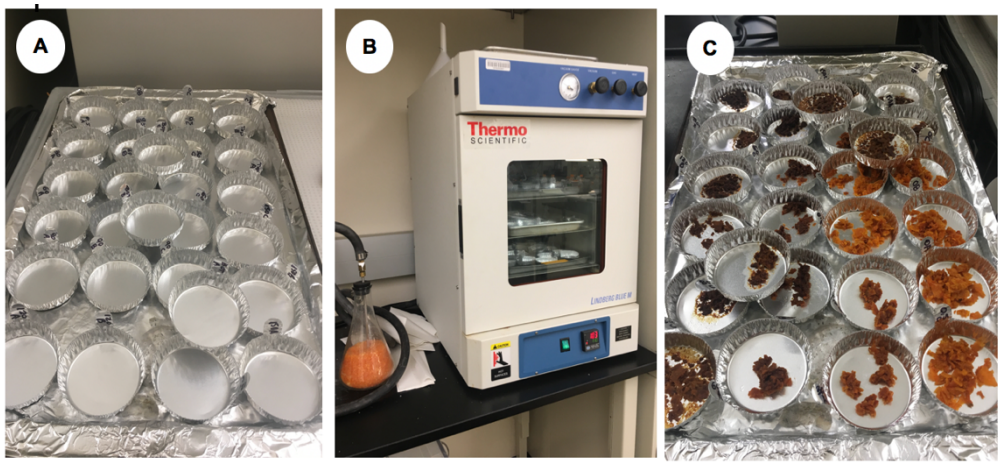

Figure 6. Labeled pans for putting samples of each timepoint (A); vacuum oven (B); samples after 20h at 110ºC (C).

3.3.3 Particle size distribution

For particle size analysis the first step was setting up the computer. After this, top (A, B, C) and bottom (A, B, C) samples were shaken in the orbital shaker variable (GeneMate, BioExpress, BT30-GM Low Speed Orbital Shaker, Model: 16020015) with deionized water for 10 min. The initial samples (0 min and 0.5 min) were imaged without addition of water. The petri dishes, each one with 1 g of sample, and the photomicrographic (PGM) scale were not overlapped to ensure that the scale was in view and relatively parallel to the edge of the image. Each top and bottom sample were divided in two petri dishes. The particles were separated using a spatula. Pictures were taken of each timepoint sample with a camera (Canon RebelSL1 EOS 100D) at height of 47 cm, avoiding glare or shadows.

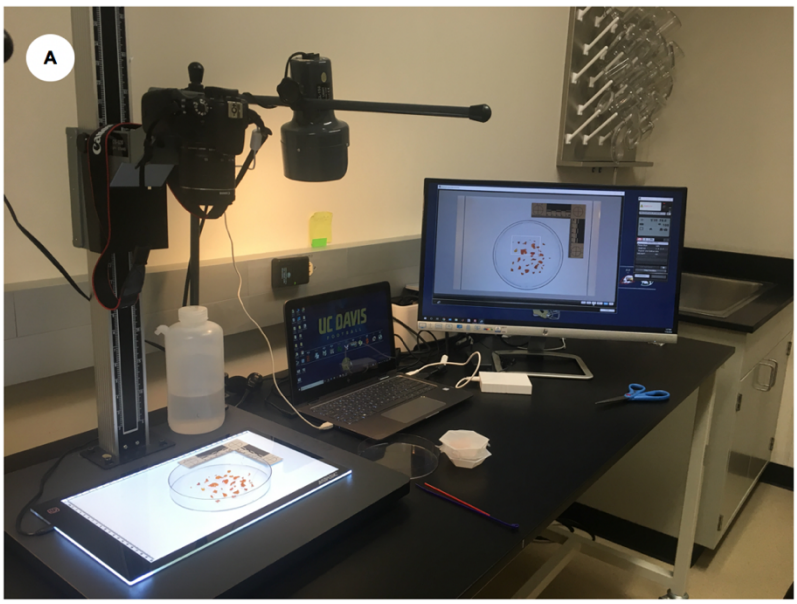

Figure 7. Needed equipment for particle size analysis (A).

3.3.4 Buffering capacity

An approximated quantity of 10 g of grounded sweet potatoes were weighed in 20 mL beakers for each sample (initial, saliva, digesta top and digesta bottom). For 1-hour digestions, 0.2 M HCl was added in aliquots of 0.5 mL to each sample (initial, saliva, digesta top and digesta bottom). For 3-hours digestions, 0.2 M HCl was added in aliquots of 0.5 mL for initial and saliva samples and was added in aliquots of 0.25 mL for digesta top and digesta bottom. After each HCl addition, the mass of potato was mixed with a spatula to homogenize the matter as much as possible and the pH was measured with a pH-meter (Dual pH Technology, IQ Scientific Instruments). The endpoint was pH 1.5 for all samples.

4. RESULTS AND DISCUSSION

Sweet potatoes are tubers that can be cooked and ingested in different ways such as fried, boiled, roasted, among others. The objective of this study was to understand the relationship between the initial food matrix, its buffering capacity, and the resulting pH gradients that occur during gastric digestion.

4.1 REGIONAL pH DISTRIBUTION

In terms of regional pH distribution, there are significant differences between 1 hour and 3 hour digestion treatments. As the diagram from Figure 8 shows, 1 hour digestion treatments pH are less homogeneous than 3 hour pH digestions.

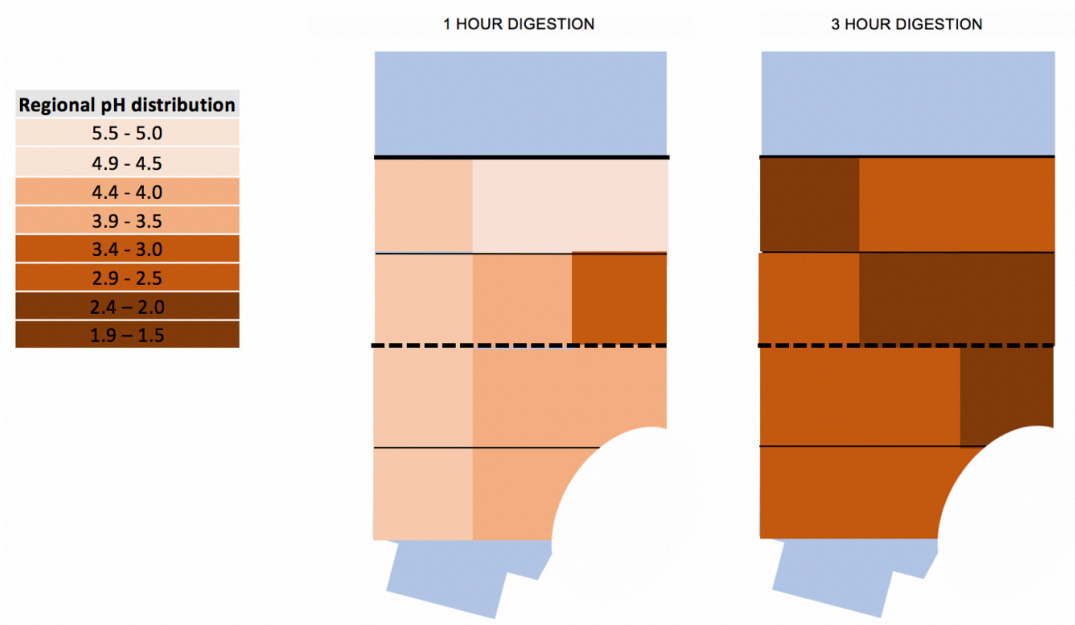

Figure 8. Color map showing regional pH distribution in fried and blanched fried treatments of 1 hour and 3 hours digestion. Each square represents the average of 12 digestions (6 of fried (three 1 hour digestions and three 3 hour digestions) and 6 of blanched-fried (three 1 hour digestions and three 3 hour digestions) with 13 individual data key points per digestion.

With the purpose of achieving the general goal, the regional pH distribution, moisture content, particle size distribution and buffering capacity were measured before and after dynamic in vitro digestion using the HGS for two cooking treatments, fried and blanched-fried. As seen in Figure 8, the regional pH distribution was different for 1 hour and 3 hour digestions in both treatments. There were so many differences between each key point in 1 hour digestions because the variability was higher in 1h than in 3h digestions: there was solid on the top of the bag, less HCl amount was secreted and HCl distribution was more irregular and, consequently, the sample was less homogeneous. Focusing in 3

hour digestions, there was more oil quantity on the top of the bag in 3h blanched-fried and fried digestions and HCl was better dissolved.

Table 1. Regional pH distribution in blanched fried and fried treatments of 1 hour and 3 hours digestion.

BLANCHED FRIED

	1 hour			3 hours		
SAMPLE	LEFT	MIDDLE	RIGHT	LEFT	MIDDLE	RIGHT
TOP - TOP	4.19 ± 0.2	5.73 ± 0.7	3.45 ± 1.4	2,45 ± 0.2	2,69 ± 0.1	2,46 ± 0.5
TOP - MIDDLE	4.31 ± 0.1	3.65 ± 1.0	2.56 ± 1.1	3,01 ± 0.8	2,42 ± 0.2	2,35 ± 0.3
BOTTOM - TOP	4.21 ± 0.1	4.05 ± 0.2	3.62 ± 0.7	2,74 ± 0.2	2,6 ± 0.2	2,40 ± 0.2
BOTTOM - MIDDLE	4.01 ± 0.1	4.10 ± 0.1	4.02 ± 0.2	2,76 ± 0.2	2,65 ± 0.2	2,49 ± 0.2
PLASTIC CAP	-	3.85 ± 0.3	-	-	2,66 ± 0.2	-

FRIED

	1 hour			3 hours		
SAMPLE	LEFT	MIDDLE	RIGHT	LEFT	MIDDLE	RIGHT
TOP - TOP	4.25 ± 1.4	5.62 ± 0.7	5.47 ± 0.7	1.55 ± 0.3	1.64 ± 0.5	1.83 ± 0.8
TOP - MIDDLE	4.06 ± 0.9	4.02 ± 0.4	3.64 ± 0.6	1.93 ± 0.4	2.27 ± 0.6	2.14 ± 1.0
BOTTOM - TOP	3.40 ± 0.8	3.77 ± 0.2	3.69 ± 0.6	2.20 ± 0.4	2.35 ± 0.3	2.38 ± 0.6
BOTTOM - MIDDLE	3.53 ± 0.7	3.79 ± 0.2	3.52 ± 0.4	2.36 ± 0.5	2.51 ± 0.4	2.43 ± 0.5
PLASTIC CAP	-	3.75 ± 0.2	-	-	2.46 ± 0.5	-

4.2 MOISTURE CONTENT

Moisture content (wet and dry basis) evolution during simulated gastric digestion for the both cooking methods is shown in Figure 9. Overall, fried and blanched-fried sweet potatoes had a greater increase in moisture over time. After 3 hours of simulated digestion: fried initial dry basis moisture content was 27.3 ± 2.4 increasing to 455.2 ± 9.6; conversely, blanched-fried initial dry basis moisture content was 33.2 ± 1.8 and it increased to 508.2 ± 16.1 g H_2O/g dry matter.

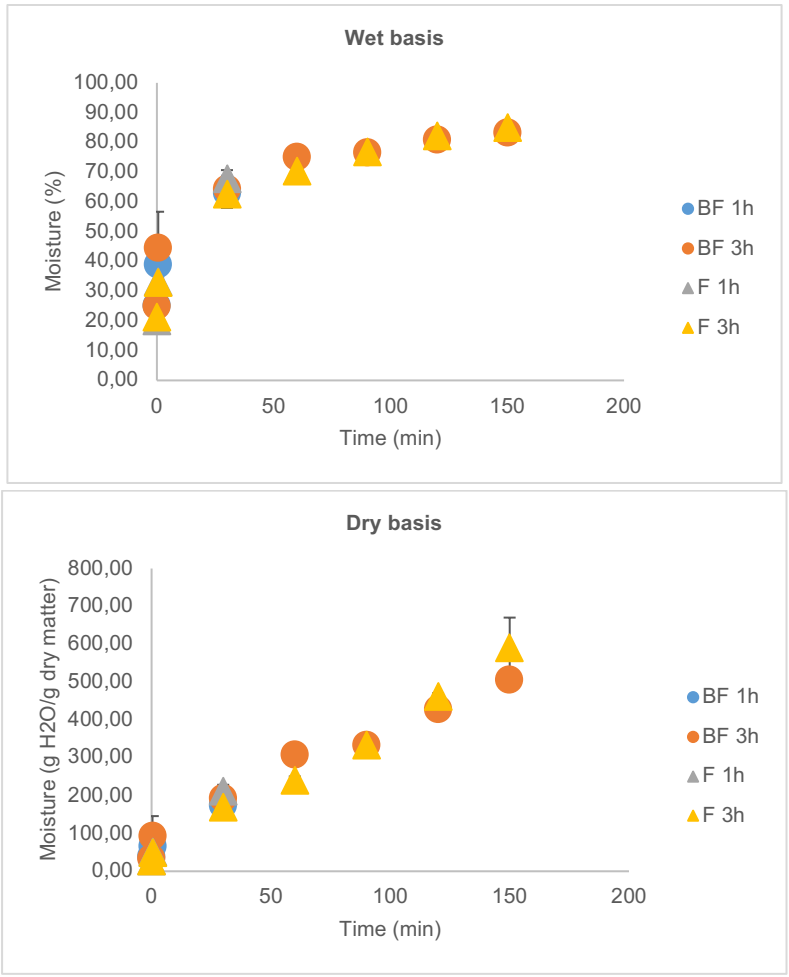

Figure 9. Moisture content (wet and dry basis) evolution during simulated gastric digestion for the two cooking methods (blanched fried and fried) of different gastric digestion duration times.

Some final samples were taken at the end of 1 hour digestions and at the end of 3 hour digestions to know their properties. The pH measurements from 0 to 150 timepoints were from material that was taken from the bag directly, nevertheless, the pH of these final samples (60 min sample and 180 min sample) was measured when digested matter was out of the bag. In other words, the bag was taken out from the HGS in 60 min and 180 min timepoints (Figure 10). Then the sweet potatoes pH was measured when digested matter was introduced in a container. In Figure 10 it is possible to see top and bottom behavior of both treatments at the end of 1 hour (60 min sample) and 3 hour (180 min sample) digestions. The results from Figure 10 were different from results in Figure 9 because the conditions in which the pH was measured changed. However, Figure 10 also shows a similar increase of the moisture content. Moreover, in these plots appear that bottom samples absorbed more water than top samples in all treatments.

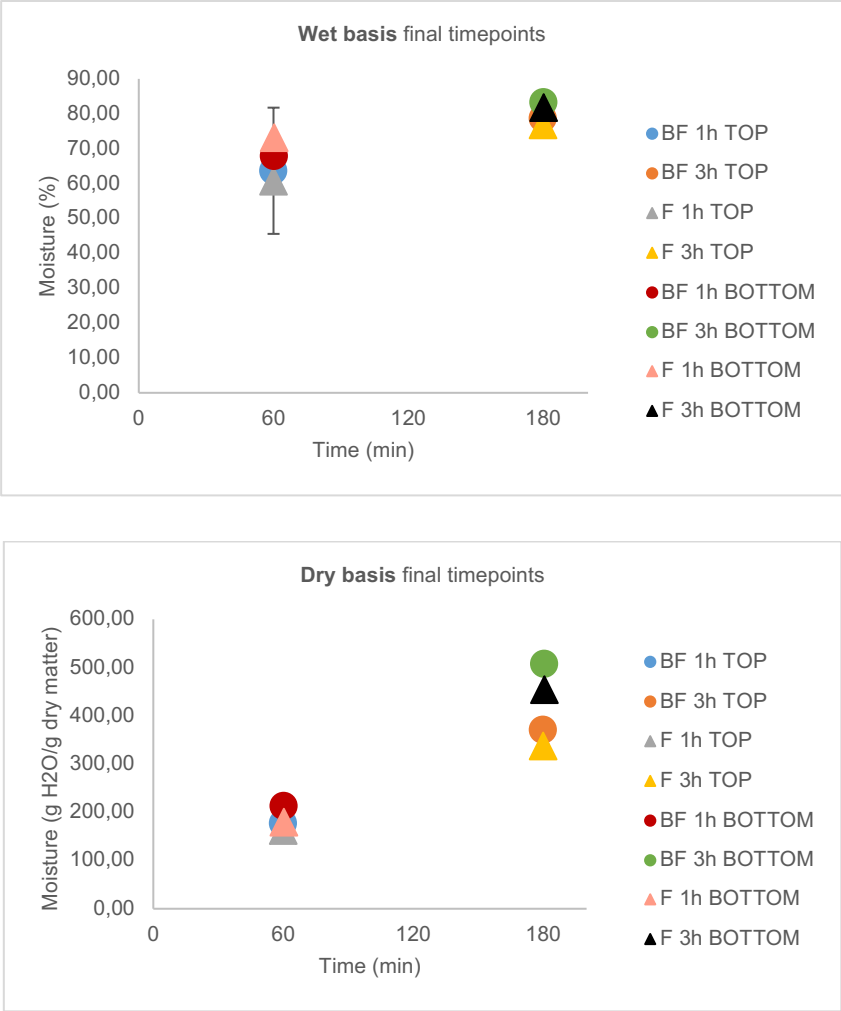

Figure 10. Moisture content (wet and dry basis) evolution during simulated gastric digestion for the two cooking methods (blanched fried and fried) of final 60 min sample and 180 min sample.

Moisture uptake in fried and blanched-fried treatments followed a similar trend as seen in Figure 9 and they didn't show significant differences. There was more variability with initial samples because the initial matter varies from one experiment to other but in the later timepoints the behavior of sweet potatoes was more consistent. The final moisture uptake was higher in 1h and 3h fried sweet potatoes than blanched fried. These results suggest that the quantity of water absorption or loss may be related with the cooking method and the food matrix.

Fat content during digestion at each location in the stomach may play a significant role for the results shown in this study. Several studies (Moreira & Barrufet, 1998; Rimac et al., 2003; Mai Tran et al., 2006; Paz, 2011; Cárdenas, 2012) have shown that the oil uptake decreases with a pretreatment, such as blanching, combined with sugar or salt solutions. Conversely, the pretreatment with only water (like in the current study) may cause more lipid absorption. The results shown in Table 1, reflect that in that case, blanching (without sugar or salt solutions) has worked and has

stopped oil absorption. Fat content for both cooking treatments from the top and bottom regions of the stomach will be analyzed in the near future to figure out if the pretreatment (blanching) is right and it acts as an oil barrier.

4.3 PARTICLE SIZE DISTRIBUTION

The evolution from particle size distribution (PSD) during the experiments is shown in Figure 11. Initial and saliva samples reflect small number of particles in PSD analysis, logical fact because they didn't suffer any digestion process. Instead of that, PSD analysis in top and bottom samples detected a greater number of particles. Frying was the treatment which had a greater number of particles in top and bottom samples.

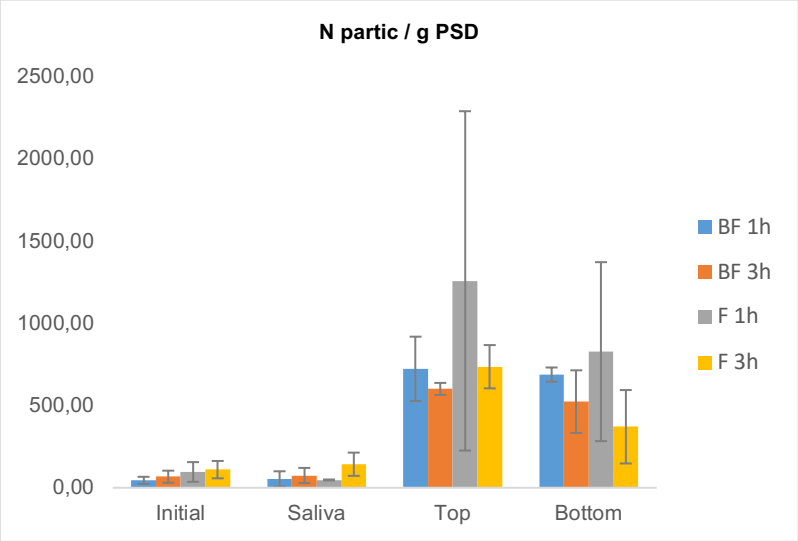

Figure 11. Number of particles per gram in initial, saliva, digesta top and digesta bottom samples for 1 hour and 3 hour digestions of blanched fried and fried treatments.

The average of the particles (x50) decreases from initial sample to bottom sample as Figure 12 represents. The results reflected in the following plot show a lot of variability between treatments.

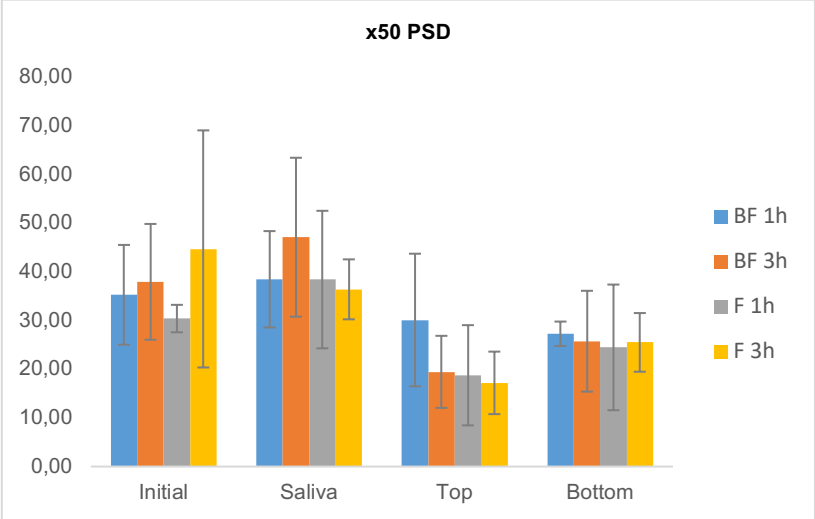

Figure 12. Medium average of particles in initial, saliva, digesta top and digesta bottom samples for 1 hour and 3 hour digestions of blanched fried and fried treatments.

The hypothesis for the number of particles per gram was that more digestion time implied a greater number of particles, because more amount of HCl was secreted into the stomach bag and due to that the breakdown rate increase. As seen in Figure 10, there were more particles in top and bottom samples than in initial and saliva samples. It makes sense because at the end of the digestion, the HCl has softened food particles and they have broken down into more small ones. Moving on now to the medium average of particles, the hypothesis was that more digestion time implied smaller particles. As Bornhorst et al. (2013) say in their study of in vivo gastric digestion, they obtained a huge percentage of particles in the proximal region so in this part, there were larger particles than particles than distal region so one of the main factors which could influence that was the digestion time. As seen in Figure 11, overall, we can see that smaller particles were in top sample in the majority of the treatments rather than in bottom sample. This fact could be explained because some huge random particles appeared in the dishes and they modified and influenced the total average.

4.4 BUFFERING CAPACITY

The addition of HCl to four samples was used to measure buffering capacity of sweet potatoes during the experiment. Initial and saliva samples had the same trend, the only difference was that the saliva initial pH was higher than in initial sample.

Conversely, top and bottom samples started to show differences between 1 hour and 3 hour treatments: 1 hour treatments needed more HCl quantity to arrive to the endpoint (pH 1.5) as Figure 13 reflects; 3 hour treatments initial pH was lower than in 1 hour digestions and, as a consequence, less quantity of HCl was needed.

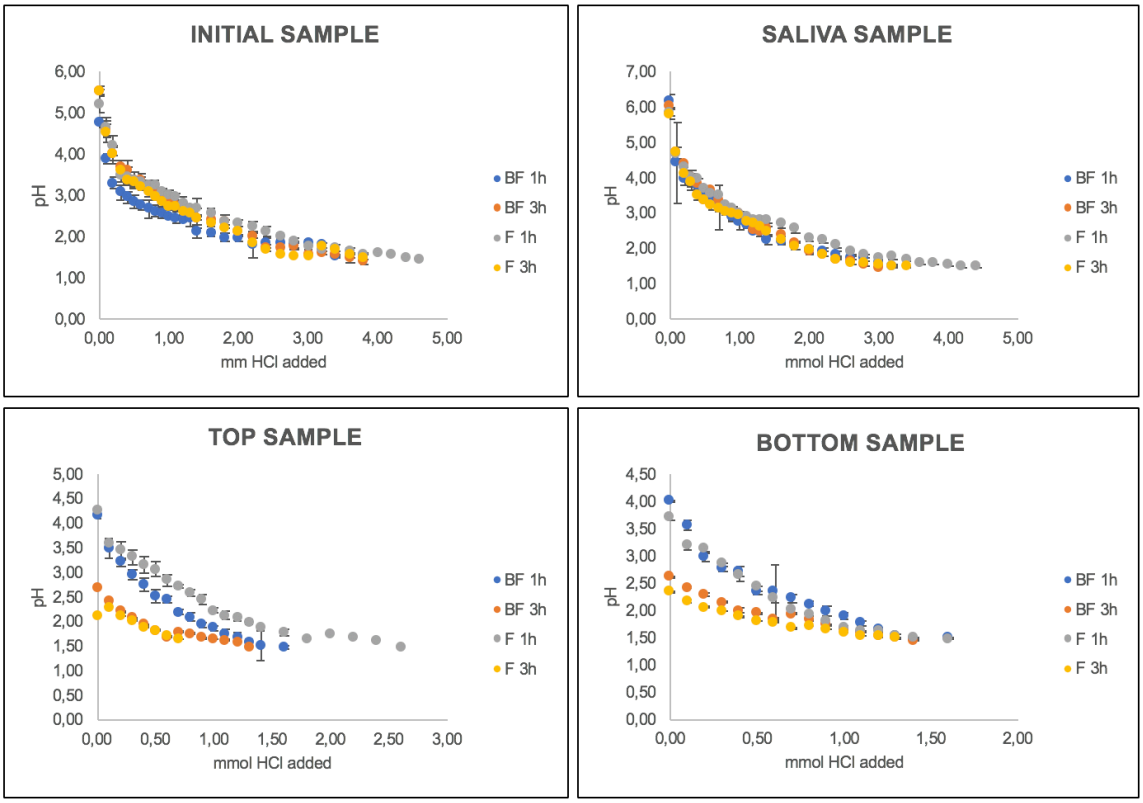

Figure 13. Buffering capacity of initial, saliva, digesta top and digesta bottom samples of two treatments digestions: blanched fried (BF) and fried (F) (n=12 digestions, 3 of each type).

Gastric environment pH is between 1.4 – 2.0 and normal acid secretion rate is around 1 mL/min to maintain this stomach pH but gastric acid secretions after ingestion increase to 6 mL/min (Malagelada, et al., 1976; Dressman at al., 1990; Barret 2005).it is hypothesized that pH in the proximal region of the stomach is higher than in the distal region because when the acid is secreted, it tends to fall down to the lower part or distal region of the stomach because of gravity force. For this reason, food bolus which is in the lower part achieves faster lower pH than food bolus which is in the upper part. So initial and saliva samples have less HCl in its matrix because the acid secretion rate is lower before digestion than in digesta samples. Top and bottom digesta samples before digestion have included more HCl in its matrix; due to that, they achieve faster the endpoint.

Besides plotting pH over mmol HCl added, other ways to represent buffering capacity to compare treatments and digestion time better are shown in the table below.

If H^+ concentration (molar) was represented vs mmol HCl added, they could show a similar trend to a microbial exponential growth behavior (lag phase and exponential growth), without considering the stationary phase. Where x axis could be the acid concentration added (mmol HCl) and y axis could be the initial hydrogen ion H concentration (molar). The parameters that will be estimated using this model will be lag phase length (lamda, λ) and growth rate (miu, μ) (Mennah-Govela, 2017).

To obtain mmol/g ΔpH data: initial mass was needed as well as ΔpH (final less initial pH of each replicate), mmol/g (obtained doing mmol added/mass) and then dividing mmol/g per ΔpH. To calculate the area under curve (AUC) an equation was needed:

$$\frac{ph1 + ph2}{2\ x\ (mmol\ added_{ph2} - mmol\ added_{ph1})}$$

Table 2. Useful parameters to represent exponential growth model of buffering capacity. In fried 180 min top and bottom the results don't appear in the table because they were negative.

Cooking method	Digestion treatment	Sample size (n)	Initial pH	AUC	Total mmol added	[H+] (molar)	mmol/g ΔpH	miu	lamda
Fried	Initial	6	5.4 ± 0.2	8.9 ± 0.3	3.5 ± 0.7	1,83E-01	88.8 ± 29.4	0.1 ± 0.1	2.3 ± 0.9
	Saliva	6	5.8 ± 0.1	9.1 ± 0.4	3.5 ± 0.5	1,89E-01	77.7 ± 17.8	0.1 ± 0.1	2.3 ± 2.3
	60 min Top	3	4.3 ± 0.1	5.1 ± 0.2	2.0 ± 0.5	8,61E-03	66.8 ± 7.0	0.0 ± 0.0	0.9 ± 0.4
	60 min Bottom	3	3.7 ± 0.1	3.2 ± 0.1	1.4 ± 0.3	5,43E-01	57.0 ± 6.4	0.0 ± 0.0	0.5 ± 0.1
	180 min Top	3	2.1 ± 0.0	1.3 ± 0.0	0.6 ± 0.6	2,73E-02	92.8 ± 9.7	0.0 ± 0.0	-
	180 min Bottom	3	2.4 ± 0.0	2.0 ± 0.0	1.0 ± 0.3	6,41E-01	114.2 ± 17.3	0.0 ± 0.0	-
Blanched Fried	Initial	6	5.5 ± 0.1	8.8 ± 0.4	3.5 ± 0.4	1,79E-01	82.8 ± 14.3	0.0 ± 0.0	1.4 ± 0.5
	Saliva	6	6.1 ± 0.2	8.0 ± 0.5	3.0 ± 0.4	2,63E-01	62.3 ± 7.9	0.2 ± 0.2	2.6 ± 2.6
	60 min Top	3	4.2 ± 0.0	3.7 ± 0.2	1.5 ± 0.1	1,07E-02	57.0 ± 9.2	0.0 ± 0.0	0.7 ± 0.2
	60 min Bottom	3	4.0 ± 0.0	3.5 ± 0.1	1.5 ± 0.1	5,11E-01	55.6 ± 6.3	0.1 ± 0.1	1.0 ± 0.4
	180 min Top	3	2.7 ± 0.0	2.0 ± 0.0	1.0 ± 0.4	1,56E-02	76.4 ± 16.0	0.1 ± 0.1	0.5 ± 1.7
	180 min Bottom	3	2.6 ± 0.0	2.3 ± 0.0	1.1 ± 0.4	6,19E-01	88.2 ± 24.8	0.1 ± 0.0	0.5 ± 0.5

5. CONCLUSIONS

This study shows the relationship between two different cooking methods, the regional pH distribution and buffering capacity of sweet potato during simulated gastric digestion. To sum up, last findings indicate that the pH varies depending on the region of the stomach and this fact is also influenced by buffering capacity of the meal. For this reason, initial and saliva samples have similar pH in 1 hour and 3 hour digestions but digesta top and digesta bottom samples pH varies between 1 hour and 3 hour digestions. Moreover, 1 hour digestions pH is more irregular because there is higher amount of solids and less HCl secretions and blanching has stopped oil uptake. Overall, moisture content shows a good rising trend in all treatments. The number of particles is bigger in top and bottom regions and the area of particles is reduced from initial to bottom sample. It is necessary to carry out the fat content analysis to gain a better oil's behavior understanding and the statistics analysis for trying to be more confident with the results. This study is important to understand the links between processing and meal properties during digestion, which will impact the physiological response to a meal.

6. REFERENCES

Ajaj, W., Goehde, S. C., Papanikolaou, N., Holtmann, G., Ruehm, S. G., Debatin, J. F., & Lauenstein, T. C. (2004). Real time high resolution magnetic resonance imaging for the assessment of gastric motility disorders. *Gut*, *53*, 1256–1261.

Barrett KE. 2005. Gastrointestinal physiology. Lange physiology series: McGraw-Hill, Columbus (OH).

Barrett KE. 2014. *Gastrointestinal Physiology*. New York: McGraw-Hill

Benini, L., Brighenti, F., Castellani, G., Brentegani, M. T., Casiraghi, M. C., Ruzzenente, O., … Vantini, I. (1994). Gastric emptying of solids is markedly delayed when meals are fried. *Digestive Diseases and Sciences*, *39*(11), 2288–2294.

Bornhorst, G. M. (2017). Gastric Mixing During Food Digestion: Mechanisms and Applications. *Annual Review of Food Science and Technology*, *8*(1), 523–542.

Bornhorst, G. M., Gouseti, O., Wickham, M. S. J., & Bakalis, S. (2016). Engineering Digestion: Multiscale Processes of Food Digestion. *Journal of Food Science*, *81*(3), R534–R543.

Bornhorst, G. M., & Paul Singh, R. (2014). Gastric Digestion In Vivo and In Vitro: How the Structural Aspects of Food Influence the Digestion Process. *Annual Review of Food Science and Technology*, *5*(1), 111–132.

Bornhorst, G. M., & Singh, R. P. (2012). Bolus Formation and Disintegration during Digestion of Food Carbohydrates. *Comprehensive Reviews in Food Science and Food Safety*, *11*(2), 101–118.

Bornhorst, G. M., & Singh, R. P. (2013). Kinetics of in Vitro Bread Bolus Digestion with Varying Oral and Gastric Digestion Parameters.

Bovell-Benjamin, A. C. (2007). Sweet Potato: A Review of its Past, Present, and Future Role in Human Nutrition. *Advances in Food and Nutrition Research*, *52*, 1–59.

Buja, A., Cook, D., Hofmann, H., Lawrence, M., Lee, E.-K., Swayne, D. F., & Wickham, H. (2009). Statistical inference for exploratory data analysis and model diagnostics. *Philosophical Transactions. Series A, Mathematical, Physical, and Engineering Sciences*, *367*(1906), 4361–4383.

Burri, B. J. (2011). Evaluating Sweet Potato as an Intervention Food to Prevent Vitamin A Deficiency. *Comprehensive Reviews in Food Science and Food Safety*, *10*(2), 118–130.

Calbet, J. A. L., & Holst, J. J. (2004). Gastric emptying, gastric secretion and

enterogastrone response after administration of milk proteins or their peptide hydrolysates in humans. *European Journal of Nutrition*, *43*(3), 127–139.

Cullen, P. J. (Patrick J. ., & Wiley InterScience (Online service). (2009). *Food mixing : principles and applications*. Wiley-Blackwell.

Donald Van Slyke, B. D. (1922). On the measurement of buffer values and on the relationship of buffer value to the dis-sociation constant of the buffer and the concentration and reaction of the buffer solution.

Emenhiser, C., Watkins, R. H., Simunovic, N., Solomons, N., Bulux, J., Barrows, J., & Schwartz, S. J. (1999). Packaging preservation of beta-carotene in sweet potato flakes using flexible film and an oxygen absorber. *Journal of Food Quality*, *22*(1), 63–73.

Feldman, M., Cryer, B., Mcarthur, K. E., Huet, B. A., & Lee, E. (1996). Effects of Aging and Gastritis on Gastric Acid and Pepsin Secretion in Humans: A Prospective Study. *Gastroenterology*, *110*, 1043–1052.

Fordtran, J. S., & Walsh, J. H. (1973). Gastric acid secretion rate and buffer content of the stomach after eating. Results in normal subjects and in patients with duodenal ulcer. *The Journal of Clinical Investigation*, *52*(3), 645–657.

Gallier, S., & Singh, H. (2012). Behavior of almond oil bodies during in vitro gastric and intestinal digestion. *Food & Function*, *3*(5), 547.

Gardner JD, Ciociola AA, Robinson M. 2002. Measurement of meal-stimulated gastric acid secretion by in vivo gastric autotitration. J. Appl. Physiol. 92(2):427-34.

Gargouri, Y., Moreau, H., & Verger, R. (1989). Gastric lipases: biochemical and physiological studies. *Biochimica et Biophysica Acta (BBA)-Lipids and Lipid Metabolism*, *1006*(3), 255-271.

Hall, J. E. (John E. (2015). *Guyton and Hall textbook of medical physiology*.

Hermansson, G., & Sivertsson, R. (1996). Gender-related differences in gastric emptying rate of solid meals. *Digestive Diseases and Sciences*, *41*(10), 1994–1998.

Hersey, S. J., & Sachs, G. (1995). Gastric acid secretion. *Physiological Reviews*, *75*(1), 155–189.

Heuman DM, Mills AS, McGuire HH Jr. 1997. *Gastroenterology.* Philadelphia: Saunders

Hoad, C. L., Marciani, L., Foley, S., Totman, J. J., Wright, J., Bush, D., … Gowland, P. A. (2007). Non-invasive quantification of small bowel water content by MRI: a validation study. *Physics in Medicine and Biology*, *52*(23), 6909–6922.

Kong, F., & Singh, R. P. (2008). Disintegration of Solid Foods in Human Stomach. *Journal of Food Science*, *73*(5), R67–R80.

Kong, F., & Singh, R. P. (2010). A Human Gastric Simulator (HGS) to Study Food Digestion in Human Stomach. *Journal of Food Science*, *75*(9), E627–E635.

Kwiatek, M. A., Fox, M. R., Steingoetter, A., Menne, D., Pal, A., Fruehauf, H., Schwizer, W. (2009). Effects of clonidine and sumatriptan on postprandial gastric volume response, antral contraction waves and emptying: an MRI study. *Neurogastroenterology & Motility*, *21*(9), 928-e71.

Lammers, W. J. E. P., Donck, L. Ver, Stephen, B., Smets, D., & Schuurkes, J. A. J. (n.d.). Origin and propagation of the slow wave in the canine stomach: the outlines of a gastric conduction system.

Low, J. W., & van Jaarsveld, P. J. (2008). The Potential Contribution of Bread Buns Fortified with β-Carotene–Rich Sweet Potato in Central Mozambique. *Food and Nutrition Bulletin*, *29*(2), 98–107.

Marciani, L., Gowland, P. A., Spiller, R. C., Manoj, P., Moore, R. J., Young, P., & Fillery-Travis, A. J. (2001). Effect of meal viscosity and nutrients on satiety, intragastric dilution, and emptying assessed by MRI.

McCabe WL, Smith JC, Harriott P. 2005.Unit operations of chemical engineering, 7th ed. NewYork, N.Y.: McGrawHill.

Meyer, J. H. (1980). Gastric emptying of ordinary food: effect of antrum on particle size. *The American Journal of Physiology*, *239*(3), G133-5.

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., … Brodkorb, A. (2014a). A standardised static *in vitro* digestion method suitable for food – an international consensus. *Food Funct.*, *5*(6), 1113–1124.

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., … Brodkorb, A. (2014b). A standardised static *in vitro* digestion method suitable for food – an international consensus. *Food Funct.*, *5*(6), 1113–1124.

Moore, J. G., Christian, P. E., Brown, J. A., Brophy, C., Datz, F., Taylor, A., & Alazraki, N. (1984). Influence of meal weight and caloric content on gastric emptying of meals in man. *Digestive Diseases and Sciences*, *29*(6), 513–519.

Moore, J. G., Datz, F. L., & Christian, P. E. (1990). Exercise increases solid meal gastric emptying rates in men. *Digestive Diseases and Sciences*, *35*(4), 428–432.

Puolanne, E., & Kivikari, R. (2000). Determination of the buffering capacity of postrigor meat. *Meat Science*, *56*(1), 7–13.

Rielly, C. D., Smith, D. L. O., Lindley, J. A., Niranjan, K., & Phillips, V. R. (1994). Mixing Processes for Agricultural and Food Materials: Part 4, Assessment and Monitoring of Mixing Systems. *Journal of Agricultural Engineering Research*, *59*(1), 1–18.

Rodriguez-Amaya, D. B., & Kimura, M. (2004). HarvestPlus Handbook for Carotenoid Analysis.

Salaün, F., Mietton, B., & Gaucheron, F. (2005). Buffering capacity of dairy products. *International Dairy Journal*, *15*(2), 95–109.

Schubert, M. L., & Peura, D. A. (2008). Control of Gastric Acid Secretion in Health and Disease. *Gastroenterology*, *134*(7), 1842–1860.

Seidel E, Long M. 2006. *Crash Course: Gastrointestinal System*. Philadelphia: Elsevier

Seok, J.W. (2011). How to Interpret Gastric Emptying Scintigraphy. *Journal of neurogastroenterology and motility*.

Sulaiman, H., Sasaki, O., Shimotashiro, T., Chishaki, N., & Inanaga, S. (2003). Effect of Calcium Application on the Growth of Sweet Potato (Ipomoea batatas Lam.) plant.

Szarka, L. A., & Camilleri, M. (2009). Methods for measurement of gastric motility.

Teow, C. C., Truong, V.-D., McFeeters, R. F., Thompson, R. L., Pecota, K. V., & Yencho, G. C. (2007). Antioxidant activities, phenolic and β-carotene contents of sweet potato genotypes with varying flesh colours. *Food Chemistry*, *103*(3), 829–838.

Wang, S., Nie, S., & Zhu, F. (2016). Chemical constituents and health effects of sweet potato. *Food Research International*, *89*, 90–116.

Williams C, Forrest A, Campbell H. 1968. Buffering capacity of food in relation to stimulation of gastric secretion. Gastroenterology 55:567-74.

Wilson, C. D., Pace, R. D., Bromfield, E., Jones, G., & Lu, J. Y. (1998). Sweet potato in a vegetarian menu plan for NASA's advanced life support program.

Woolfe, J. A. (1992). *Sweet potato: an untapped food resource*. Cambridge University Press.

Wu, D., Lu, J., Zheng, Y., Zhou, Z., Shan, Q., & Ma, D. (2008). Purple sweet potato color repairs d-galactose-induced spatial learning and memory impairment by regulating the expression of synaptic proteins. *Neurobiology of Learning and Memory*, *90*(1), 19–27.

7. ANNEXES

7.1 ANNEXE A. Cooking Method protocol

Raw materials preparation and processing

Two different snacks were designed from the raw potatoes provided by the company. Sweet potato's healthy snacks are: fried, baked, blanched & fried, and blanched & baked. Each product has its own cooking method. This are developed in the next section.

All of them share the raw material preparation method. The sweet potatoes are cleaned with water for removing all the dust remanding on the skin. Then, they are pealed by hand with a potato's peeler. The end of the potatoes (both sides of the potato) is removed. Finally, they are cut with a meat slicer with 3 mm thick. About 300g are needed for each batch of cooking for obtaining approximately100g of cooked potatoes.

For each digestion trial, 200g of processed sweet potatoes is needed.

1. Clean sweet potatoes with water.
2. Peal it and cut ends of the potato.
3. Slide it 3mm.
4. Weight about 300 g for processing

Cooking methods

Fried

The fryer is filled with new soybean oil. It is warmed to 340ºF. Once the oil has the appropriate temperature, 300g of sweet potatoes is introduced in a basket into the oil. They are cooked for 5 min 30s. When the cooked time has elapsed, take the basket off of the oil and let it dry for 10 min in the basket. Then put them in a tray with paper towels.

Preparation

1. Put new oil.
2. Warm oil to 340ºF.

Frying

1. Put 300g sweet potatoes in the basket.
2. Introduce the basket in the oil.
3. Cooked it for 5min 30s.
4. Take the basket off of the oil, let it dry for 10 min in the basket.
5. Put them in a tray with paper towels.

Blanching

For those products that needs a blanching before the main cooking procedure. Put one liter of water in a pot and warm it to 85ºC. Once the water achieved the temperature, out 150g of sweet potatoes in the pot. Cook them for 1 min. Then, immediately take them out of the hot water and put on a bowl with cold water during a minute. Once they are cooled, put them in a new tray with paper towels during 5 min. Then, fry or baked it following the instructions given before.

Preparation

1. Warm water in a pot to 85ºC.

Baking

1. Put 150g sweet potatoes in the pot.
2. Cooked it for 1 min.
3. Take the sweet potatoes off of the pot, put them immediately into cold water and let it cool for 1 min.
4. Put them in a new tray with paper towels during 5 min.
5. Fry or bake them.

7.2 **ANNEXE B.** Human Gastric Simulator protocol

Buffering Capacity HGS Sweet Potato Digestion Protocol

1. **Put moisture pans in the oven**
2. **Prepare fluids**
   - Calibrate pH-meter
   - Prepare lipase solution
     - 3h digestion →130 mL + weigh 3.03g of lipase
     - 1h digestion → 65 mL
     - Adjust lipase pH to 4.5
   - Measure gastric juice
     - 3h digestion → 700mL + put it in the water bath
     - 1h digestion → 235 mL + put it in the water bath
     - Adjust gastric juice pH to 0.8
     - Add enzyme:
       - ✓ 3h digestion → 5.45g of pepsin (for 700 mL)
       - ✓ 1h digestion → 1.8296g of pepsin (for 300 mL)
   - Measure saliva
     - 3h digestion → 60 mL
     - 1h digestion → 60 mL
     - Adjust saliva pH to 7
     - Add enzyme:
       - ✓ 3h digestion → 0.071g of alpha-amylase (for 60 mL)
       - ✓ 1h digestion → 0.024g of alpha-amylase (for 20 mL)

3. **Cook sweet potatoes**

   - Clean sweet potatoes with water and peal them
   - Slice sweet potatoes with the meat slicer with a thickness of 3 mm
   - Weigh 300 g of sweet potatoes, 3 times to have a total of 900 g of raw sweet potatoes
   - Cook the potatoes
     - Frying
       - New soybean oil (~3.5 L)
       - Temperature 340 ºF
       - Time 5 min 30 seconds
       - Cool 20 min
     - Blanching
       - 1L tap water
       - Temperature 85 ºC
       - Time 1 min
       - Dry 10 min

4. Initial properties

— While cooling, prepare stomach bag
— Set up HGS with person 2.
— Process the chips (~300 g) in the food processor during 45s high frequency.
— Weigh quantity necessary for digestion:

$$200\ g\ dry\ matter\ \times \%\ moisture$$

- 208g for fried (4%)
- 216g for blanched-fried (8%)

— Mix processed potatoes with the saliva

$$g\ sample\ \times 0.2\ {}^{ml\ saliva}/_{g\ sample}$$

- 208g x 0,2 = 41,6 mL
- 216g x 0,2 = 43,2 mL

— **Prepare HGS**

- Turn on heating lamp
- Put the bag
- Add 70 mL of gastric juice into the bag
- Fill out pumps
  - Peristaltic pump (gastric juice): 3.0 mL/min
  - Syringe pump (lipase): 0.5 mL/min
- Start HGS with Natalia
  - Mix processed food with saliva during 30s
  - Introduce processed food into the bag
  - Collocate stomach bag in the right position with tape

— **START GASTRIC DIGESTION**

- During first 30 min of gastric digestion:
  - Processed chips (0 min)
    - Measure pH
    - Measure moisture content (3 pans)
    - Prepare for particle size (3 samples)
    - Freeze (2 tubes)
  - Processed chips + saliva (0.5 min)
    - Measure pH
    - Measure moisture content (3 pans)
    - Prepare for particle size (3 samples)
    - Freeze (2 tubes)

- Sample of the bag (each 30 min)
    - Weigh the bottle before taking the sample
    - Measure pH
    - Measure moisture content (3 pans for each timepoint)
- Final digestion time
    - Measure pH of the bag (point 6)
    - Measure moisture content (3 pans for top and 3 for bot)
    - Freeze (2 tubes for top and 2 tubes for bottom)

5. Measure buffering capacity

— Initial properties
    - Buffering capacity processed potatoes
    - Buffering capacity processed potatoes + saliva

— Final properties
    - Buffering capacity sample last point of gastric digestion: TOP and BOTTOM

6. Measure bag pH distribution

7. Particle size

a) Set up the computer.
b) Shake the samples in the shaker with some water during 10 min
    a. To the initials that has not much moisture, do not add water.
c) Each sample is divided in two dishes.
d) Separate the particles with a spatula.
e) Take the picture.

7.3 **ANNEXE B.** Particle Size Distribution protocol

Particle Size Protocol

Criteria for good images

*** Setting up the stage**

1. **The photomacrographic (PMG) scale is in view, and relatively parallel to the edge of the image**

    a) This PMG scale is the thing with the lines on it that looks like an L-shaped ruler

2. **The image has no obvious glares or shadows**

    a) If you find it has glare or shadows, you can always adjust the lighting and capture it again.

    b) The optimal lighting seems to be when the ceiling lights are on (but not the red ones), the lightbox set to the brightest setting, and the stage lights (the two yellow ones) pointing straight down, but with the shielded side facing the stage. The stage is the place where the petri dish sits.

3. **The petri dish and the PMG scale are not overlapping**

4. **The almond particles in the petri dish are not overlapping.**

5. **The big petri dish is used**

    a) Double check that the edge is highlighted in black marker. If it is not, use a marker to outline it again. This helps the code find the edge of the dish.

6. **Camera at height of 47cm**

A good example image is shown below:

Note that the outer edge of the dish is darkened with marker. Also note how there are no glares, shadows, objects halfway out of the image, or objects overlapping. Also note that none of the particles are overlapping each other.

*** Using the camera**

1. Connect the camera to the computer using the white, micro HDMI cable.
2. It connects to the camera in a port that is under a rubber flap.
3. Take off the lens cap
4. Turn the dial on the top of the camera to “On”
5. Turn the dial so that the white line goes to “M”
    a. This is the manual setting, which is the one we want
6. Go to the computer
    a. Password is “OdRbtydb*n*”
7. Go to “EOS Utility”
8. Select “control camera”
9. Select “camera settings and remote shooting”
10. Click on “Live view shoot” near the bottom of the control panel
11. Double check that the stuff in the image is all good (see previous section)
12. Double check that the settings are “1/30”, “F8.0”, “ISO 100”, “AWB”

13. In the window that shows the live camera view, click on the third button from the right and make sure that “Aspect ratio” is 4:3
14. Right below the round, circular button on the top is a folder button. Click it and make sure that the images are being saved to the correct folder. In the same window is a box called “Download images,” which should have a check mark.
15. Click the round, circular button near the top to take the photo